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THE URINARY EXCRETION OF NORADRENALINE AND ADRENALINE IN DIFFERENT AGE GROUPS, ITS DIURNAL VARIATION AND THE EFFECT OF MUSCULAR WORK ON IT

BY

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PREFACE.

The experimental work described in this publication has been carried out at the Department of Pharmacology of the University of Turku. I am very much indebted to Professor Aimo Pekkarinen, M.D., the Head of the Department, for suggesting the subject and for his continued interest and encouragement during the course of this work. I have been very fortunate in being able to benefit from his wide experience in this field of study.

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Turku, September 1956.

N. T. Kärki.

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I

INTRODUCTION.

The work that has been done during the last ten years has greatly contributed to our knowledge of the function of noradrenaline and adrenaline as transmitters of the sympathetic postganglionic nerves and as hormones of suprarenal medulla.

The studies of HOLTZ, CREDNER and KRONEBERG (1947) and v. EULER and HELLNER (1951) which revealed that noradrenaline and adrenaline can be quantitatively estimated in urine showed that it is possible to obtain information about the liberation of these compounds in the organism by determining the urinary excretion. Only relatively few studies have yet been published in which the normal excretion of noradrenaline and adrenaline in man has been evaluated on the basis on extensive material and hence it is not yet possible to draw reliable conclusions from determinations made for diagnostic purposes (FORSSMAN 1954).

The present study is concerned with the excretion of noradrenaline and adrenaline in man, especially as far as this is influenced by age and sex. Also the effect of physical exertion on the excretion of noradrenaline and adrenaline in urine has been followed in subjects engaged in various sports and in heavy muscular work.

The noradrenaline and adrenaline contents of urine have been evaluated by determining their biological action on the blood pressure of the cat and rat and on the hen's rectal caecum.

Since the extraction methods employed in the preparation of samples for the biological determination were involved and time-consuming when the present investigation was begun in 1953, a more simple method has been developed which shortens the time required for the performance of the determinations. A preliminary report dealing with some of the results of the present investigation was published by the author in the Acta Physiologica Scandinavica in 1954.

II

LITERATURE REVIEW.

A. FUNCTION OF NORADRENALINE AND ADRENALINE AS MEDIATORS OF THE SYMPATHETIC NERVOUS SYSTEM.

The present view that the function of the sympathetic nervous system is based on a chemical mediator which is liberated at the nerve endings and activates the receptor system was proposed by ELLIOT in 1904. He concluded that the chemical mediator was adrenaline. In 1921 LOEWI as well as CANNON and URIDIL contributed greatly to our knowledge of the significance of humoral factors in the function of the sympathetic nervous system. LOEWI found that stimulation of the cardiac nerves of the frog results in the liberation of a sympathomimetic substance which he later (1936, 1937) identified as adrenaline. The latter authors, CANNON and URIDIL, observed that stimulation of the sympathetic nerves provoked a response in the denervated heart of a cat from which the suprarenal glands had been removed. They also noted that the effect on the size of the pupils differed clearly from that brought about by adrenaline. Also earlier, in 1910, BARGER and DALE in their systematic study of sympathomimetic compounds had doubted that the substance liberated by nerve stimulation in mammals is adrenaline. Their opinion was that adrenaline has a stronger inhibiting effect than nerve stimulation and that the amounts of ergotoxin required to prevent the action of adrenaline were much smaller than those required to suppress the effect of nerve stimulation. Several theories were subsequently advanced to explain the properties of sympathin, which name CANNON and BACQ (1931) gave to the assumed transmitter substance. On the basis of their

studies, CANNON and ROSENBLUETH (1933, 1935) proposed a hypothesis according to which sympathin combines with various receptors and that, depending on the receptor, the combination product may either be an exciter, sympathin E, or an inhibitor, sympathin I. In 1934 BACQ concluded that the strong excitatory properties of sympathetic nerve stimulation are due to liberated noradrenaline. Several later experimenters supported his view (STEHLE and ELLSWORTH 1937, GREER, PINKSTON, BAXTER and BRANNON 1938).

The investigations of v. EULER (1946 a, b, c, d) in which he showed that the tissue extracts of various organs contained a sympathomimetic substance which possessed similar biological and chemical properties as noradrenaline and his observation that the sympathetic nerves contained a substance closely resembling noradrenaline provided the foundation for the opinion that the primary transmitter in mammals is noradrenaline. In 1947 BACQ and FISCHER proposed that sympathin should be taken to denote the mixture of noradrenaline and adrenaline which is secreted by the adrenergic nerves. Soon information was forthcoming as to the amounts of noradrenaline and adrenaline present in the extracts of nerves and tissues (v. EULER 1948 a, b, 1949 a, b). Also many other investigators found that both noradrenaline and adrenaline are present in all parts of the organism, although in varying amounts, and that their secretion can be effected by nerve stimulation (e.g. GADDUM and GOODWIN 1947, SCHMITERLÖW 1948, GOODALL 1950, PEART 1949, MANN and WEST 1950, HÖKFELT 1951).

The close connection between noradrenaline and adrenaline and the nervous system is also indicated by the observation that if nerve tissue is absent, as in the placenta, these catechol amines cannot be isolated from the organ (v. EULER 1945). Further evidence is provided also by SCHMITERLÖW's observation (1948) that the noradrenaline content of the wall of a blood vessel is proportional to the density of nerve fibres in it.

B. NORADRENALINE AND ADRENALINE AS HORMONES OF THE SUPRARENAL MEDULLA.

At the same time as the function of noradrenaline as the main transmitter of the sympathetic nervous system was clarified, it was discovered that it appears together with adrenaline as a hormone of the suprarenal medulla.

The fact that the suprarenals contain appreciable amounts of a sympathomimetic substance was established at an early date, when OLIVER and SCHÄFER (1895) found that extracts of suprarenal glands are able to increase the blood pressure of test animals by contracting the blood vessels and raising the heart rate. In 1901 TAKAMINE isolated the active substance, which was named adrenaline, in crystalline form. In 1904 STOLZ effected the synthesis of adrenaline and also of noradrenaline. Adrenaline was long considered to be the only sympathomimetic substance produced by the suprarenal medulla, although observations had been made that in some cases suprarenal gland extracts had a stronger action on the blood pressure than could be ascribed to the colorimetrically estimated amounts of adrenaline present in the extracts (SCHILD 1933). HOLTZ, CREDNER and KRONEBERG (1947) were the first to suggest that the suprarenal gland contains noradrenaline in addition to adrenaline. They observed that the reaction of the blood pressure of a cat to suprarenal gland extracts after yohimbin was similar to that effected by a mixture of noradrenaline and adrenaline. On the basis of the effect of bovine suprarenal gland extracts on blood pressure and blood sugar level, HOLTZ and SCHÜMANN (1948) and SCHÜMANN (1949 a, b) concluded that noradrenaline was responsible for 25 per cent of the sympathomimetic activity of the extracts. v. EULER and HAMBERG (1949), GADDUM and LEMBECK (1949), HOLTZ and SCHÜMANN (1949, 1950) and WEST (1950) confirmed this conclusion. BERGSTRÖM, v. EULER and HAMBERG (1949, 1950) succeeded in isolating noradrenaline in crystalline form from suprarenal extracts, and TULLAR (1949) from U.S.P. epinephrine.

The relative amounts of noradrenaline and adrenaline in the suprarenal glands vary greatly in different animals (HÖKFELT 1951, SHEPHERD and WEST 1951 a, b, ERÄNKÖ 1955). These authors

and also HOLTZ and SCHÜMANN (1950) found that the catechol amines of the suprarenal glands of a full-grown rabbit consist almost solely of adrenaline. The major part of the catechol amines in embryonal suprarenals of many animal species is composed of noradrenaline. The same is also true for the human embryo. At birth, the percentage of noradrenaline is 70, but it decreases with age (HÖKFELT 1951, SHEPHERD and WEST 1951 a, b). NIEMINEVA and PEKKARINEN (1952) showed that in three-fourths of fetuses weighing from 230 to 4270 g more than half of the total catechol amine content was adrenaline. ERÄNKÖ (1956) found that the ratio of adrenaline and noradrenaline is the same in the suprarenal glands of both full-grown and embryonal rats.

The suprarenal glands of adult humans who have suffered accidental deaths have been found to contain 0.72 mg of adrenaline per gram of tissue (UOTILA and PEKKARINEN 1951). The contents of adrenaline and noradrenaline in suprarenal glands removed by surgery have been found by v. EULER, FRANKSSON and HELLSTRÖM (1954 b) to be, respectively, 0.48 mg and 0.09 mg per gram of tissue, the adrenaline amounting to 84 per cent of the total catechol amines in the glands.

In recent years the distribution of noradrenaline and adrenaline in the cells of the suprarenal glands of different species has been studied by a histochemical fluorescence method (ERÄNKÖ e.g. 1952, 1955) and by utilizing the iodate reaction (HILLARP and HÖKFELT 1953, 1954).

HOLTON (1949 a, b) found that tumors of the suprarenal gland, phaeochromocytomas, may contain large amounts of noradrenaline and adrenaline. This finding has been corroborated by many authors (e.g. GOLDENBERG et al. 1949, ENGEL and v. EULER 1950).

C. THE BIOLOGICAL PROPERTIES OF NORADRENALINE AND ADRENALINE.

Noradrenaline and adrenaline are known to differ in their biological properties. Adrenaline has a clearly stronger inhibitory effect than noradrenaline on many organs (GADDUM, PEART and VOGT 1949, WEST 1947). Noradrenaline, however, is more effective

in increasing the blood pressures of animals (Tainter et al. 1948, Graham 1949). Ahlquist et al. (1954) showed that noradrenaline produces greater increases in the systolic, diastolic and mean blood pressures than adrenaline in dogs anesthetized with pentobarbital, that but its contracting effect on the bladder, intestine and spleen is weaker than that of adrenaline. Adrenaline in small doses dilates the blood vessels, while noradrenaline always causes vasoconstriction in cat muscle (Folkow et al. 1948). On the other hand, noradrenaline dilates the coronary vessels (Folkow et al. 1949, Burn and Hutcheon 1949). Small amounts of noradrenaline increase the blood flow through muscle also in humans (Grant and Pearson 1938, Allen et al. 1946, Barcroft and Konzett 1949, Barcroft and Cobbolt 1956). Noradrenaline effects vasoconstriction, increased diastolic, systolic and mean blood pressures and bradycardia, whereas adrenaline increases cardiac output and systolic blood pressure without affecting appreciably the mean blood pressure, decreases peripheral resistance and accelerates the heart (Goldenberg et al. 1948, Barcroft and Konzett 1949, Bearn et al. 1951, Pekkarinen and Hortling 1951, Helve and Pekkarinen 1952).

In animals adrenaline has been found to be more effective than noradrenaline in increasing the oxygen consumption (Thibault 1948, Lundholm 1949). According to Schümann (1949 a) noradrenaline is only one-tenth as active as adrenaline in increasing the blood sugar level in rabbits, while Bloom and Russel (1952) reported that the effect of subcutaneously administered noradrenaline on the blood sugar level in the rat is only one-third of that of adrenaline.

Also in humans it has been observed that, in contrast to adrenaline, noradrenaline has very little effect on the oxygen consumption (Goldenberg 1951, Bearn et al. 1951, Helve and Pekkarinen 1952). Neither has noradrenaline been observed to increase the lactic acid content of human blood, as adrenaline has been found to do (Bearn et al. 1951, Helve and Pekkarinen 1952). Helve and Pekkarinen also found that adrenaline reduces the inorganic phosphate content of the blood, whereas the effect of noradrenaline is very slight.

D. DETERMINATION OF NORADRENALINE AND ADRENALINE IN THE BLOOD.

The liberation of adrenaline and noradrenaline in the animal organism through the action of nerve stimulation or various other stimuli can be followed by comparing the reactions of the blood pressure, the iris, the nictitating membrane and other sensitive organs *in situ* with those produced by the pure compounds.

The concentrations of noradrenaline and adrenaline in the suprarenal venous blood of experimental animals are frequently so high that the noradrenaline and adrenaline can be directly estimated in the plasma using various sensitive test organs (Gaddum and Lembeck 1949, West 1950, Kaindl and v. Euler 1951, Brücke et al. 1952, Outschoorn 1952, Duner 1953, v. Euler and Folkow 1953, Folkow and v. Euler 1954). The concentrations can also be evaluated by chemical methods (Pekkarinen 1948, Lund 1951 b).

In several investigations, Japanese workers have thoroughly studied the effect of various factors on the secretion by the suprarenal glands in experimental animals, but they have only determined the adrenaline content in the suprarenal venous blood (for references, see Satake 1954).

The determination of noradrenaline and adrenaline in the peripheral blood is much more difficult, even in test animals (Mann and West 1950). Owing to the low contents of noradrenaline and adrenaline, attempts have been made to separate these compounds from other substances that may lead to erroneous results. Using paper chromatography, Outschoorn and Vogt (1952) have found the blood from the coronary sinus of a dog to contain 1.0 to 1.5 μg of adrenaline and 2.5 to 10 μg of noradrenaline per litre. Vogt (1952) analysed 1 to 6 μg of adrenaline per litre in the arterial blood of rats anesthetized with ether. Mirkin and Bonnycastle (1954) observed that stimulation of the postganglionic sympathetic nerves of the liver and spleen caused the release of sympathomimetic substance consisting of 90 per cent noradrenaline and 10 per cent adrenaline into the blood. Holzbauer and Vogt (1954) found the adrenaline content in the peripheral blood to vary from 0.25 to 6.4 μg per litre of plasma during insulin hypoglycemia in dogs.

The noradrenaline and adrenaline present in the peripheral blood of human subjects have also been estimated. PEKKARINEN (1948) found by means of a fluorimetric method that the adrenaline content of the peripheral blood was less than the limit of sensitivity of the method, 0.5 μg per 100 ml. Using a biological method, v. EULER and SCHMITERLÖW (1947) found the noradrenaline content of the blood to vary from 1 to 2 μg per 100 ml, but v. EULER (1952) later considered this estimate to be too high. Both the biological and chemical methods are frequently too non-specific (FLEETWOOD 1951, HELLER et al. 1951). Using the ethylene diamine reaction, WEIL-MALHERBE and BONE (1953) found the plasma of healthy human beings to contain 0.52 μg of noradrenaline and 0.1 to 0.15 μg of adrenaline per 100 ml. WEIL-MALHERBE (1953) observed also that insulin lowers the content of adrenergic amines in the blood. HOLZBAUER and VOGT (1954) found using a biological method that the adrenaline content of human venous blood is less than 0.006 μg per 100 ml and increases to 0.18 μg per 100 ml during insulin hypoglycemia. They were unable to detect noradrenaline in the blood with their method which had a sensitivity of 0.1 μg per 100 ml.

Noradrenaline and adrenaline are present in larger amounts in the blood of cases of phaeochromocytoma and of paraganglioma than in normal blood. Using LUND's (1949, 1950) adsorption procedure and fluorimetric determination, LUND and MÖLLER (1951), LUND (1952) and v. EULER, LUND, OLSSON and SANDBLOM (1953) performed analyses on such subjects. During attacks, the contents of these compounds were observed to rise to the level of a few micrograms per 100 ml of blood.

It must be noted that despite the results that have been obtained when attempts have been made to estimate noradrenaline and adrenaline in human peripheral blood, no simple, reliable method is yet available that can be used to follow the variation of these compounds in the organism by examining their contents in peripheral blood. Exceptions are those conditions in which the secretion of these substances is greatly increased. It should further be remembered that adrenaline and noradrenaline rapidly disappear from the blood (PEKKARINEN 1948, LUND 1951 a).

E. EXCRETION OF NORADRENALINE AND ADRENALINE IN THE URINE.

In 1909 FALTA and IVCOVIC were able to obtain the reaction typical of adrenaline when they added ferric chloride to the urine of a subject who had been given adrenaline orally. Also RICHTER (1940) and BEYER and SHAPIRO (1945) found by chemical methods that orally administered sympathomimetic substances, including adrenaline, are excreted in the urine.

HOLTZ, CREDNER and KRONEBERG (1947) were the first to establish the presence of biologically active catechol amines in normal human urine. Using different biological methods they were able to show that the sympathomimetic substance which they had isolated from the urine and which they called urosympathin was composed of oxytyramine, adrenaline and noradrenaline. v. EULER and HELLNER (1951) confirmed this result in a study of the urine from a group of twenty healthy young persons. The mean daily excretion of noradrenaline they found to be about 30 μg, and that of adrenaline about 11 μg. Primarily owing to the use of different methods for treating the urine and for determining the amine contents, figures for the normal secretion have been reported later that differ appreciably from these. Using fluorimetric methods, PEKKARINEN and PITKÄNEN (1955 b) have found the daily urinary excretion of noradrenaline by adults to vary from 50 to 100 μg. PITKÄNEN (1956) established that the total amount of noradrenaline excreted is 68 μg and that of unconjugated noradrenaline 33 μg, the adrenaline percentage being 29. A daily excretion of noradrenaline not exceeding 50 μg as determined by biological methods is considered normal (BURN 1953, WEST 1954, GOLDENBERG et al. 1954, v. EULER, HELLNER and PURKHOLD 1954).

Also the effect of various physiological and pathological conditions on the excretion of noradrenaline and adrenaline in the urine has been studied. v. EULER and HELLNER (1952) found that the amounts of adrenaline and noradrenaline excreted in the urine were greatly increased by heavy muscular work.

Emotional stress has been observed to increase primarily the urinary secretion of adrenaline, as shown by v. EULER and LUNDBERG (1954) in the case of pilots.

Variations in hormonal equilibrium and administration of various hormones alter the adrenaline and noradrenaline concentrations in the urine. v. EULER and LUFT (1949) and LUFT and v. EULER (1952) found that administration of ACTH and cortisone lowers the noradrenaline content of the urine appreciably with the result that the relative amount of adrenaline increases since the latter undergoes only a slight change. Insulin has been observed to increase greatly the excretion of adrenaline in the urine; in some cases the increase may be as much as tenfold in man (v. EULER and LUFT 1952) and even twentyfold in rats (PITKÄNEN 1956). RAAB and GIGEE (1954) have found by a fluorometric method that the amounts of catechol amines are much below the normal in cases of hypopituitarism.

In stress conditions in man resulting in death, UOTILA and PEKKARINEN (1951) have observed that activation of the suprarenal cortex is accompanied by clear signs of increased excretion of noradrenaline and adrenaline by the suprarenal gland. In 27 surgical patients, FRANKSSON et al. (1954) established augmented cortical activity which in some cases was associated with a large excretion of noradrenaline and adrenaline in the urine, which signifies activation of the adrenergic nervous system or of the suprarenal medulla. HALME, PEKKARINEN and TURUNEN (1956) studied the effect of major surgical operations on the amounts of 17-ketosteroid and 11-hydroxycorticosteroid hormones, adrenaline and noradrenaline excreted in the urine in a series of 100 patients, and found that the amounts of adrenaline and especially of noradrenaline and the steroid hormones excreted in urine underwent substantial increases during 2—3 days following the operations. The excretion of noradrenaline and adrenaline has also been observed to increase in cases of myocardial infarction (NUZUM and BISCHOFF 1953, FORSSMAN 1954, RAAB and GIGEE 1954). One of the problems involved when studying the excretion of adrenaline and noradrenaline in the urine is the relation between the excretion of noradrenaline and circulatory disorders. The excretion of noradrenaline and adrenaline has been found to be very low in postural hypotension (LUFT and v. EULER 1953).

In cases of phaeochromocytoma, on the other hand, which are characterized by high blood pressures or at least hypertonic attacks, the contents of noradrenaline and adrenaline in the urine have

been found to greatly exceed the normal. ENGEL and v. EULER (1950) introduced this observation as an aid in the diagnosis of the disease. Many cases have been described which have been diagnosed by estimating the noradrenaline and adrenaline content of the urine: v. EULER (1951) 6 cases, HAMILTON et al. (1953) 5 cases, GOLDENBERG et al. (1954) 16 cases, and PITKÄNEN (1956) 5 cases. Nowadays an increased content of noradrenaline and adrenaline in the urine as determined by chemical or biological methods is considered the most reliable diagnostic criterion of this disease. The best results are obtained if the urine is collected during an attack. The total amount of adrenaline and noradrenaline secreted varies from 100 μg to 4000 μg per 24 hours, and the relative amounts of the two substances may vary within wide limits.

The urinary excretion of noradrenaline in urine in cases of essential hypertension has also been investigated. HOLTZ, CREDNER and KRONEBERG (1947) reported that some of the patients may excrete 2—3 times the normal amount of noradrenaline. This observation has not been verified in studies made by GOLDENBERG and RAPPORT (1951), GOLDENBERG et al. (1954), HAMILTON et al. (1953), BURN (1953), RAAB and GIGEE (1954), and WEST (1954). WEST examined 50 hypertensive patients and found an increased secretion in only one case, and even this case was later found to suffer from phaeochromocytoma. No clear increase in noradrenaline excretion was detected by a chemical method in 25 cases of hypertension by PEKKARINEN and PITKÄNEN (1955 b) or in a series of 350 cases examined by PITKÄNEN (1956) who were suspected to suffer from phaeochromocytoma. Using a biological method, v. EULER, HELLNER and PURKHOLD (1954) evaluated the 24-hour excretion of noradrenaline in urine in 500 patients suffering from high blood pressure. They came to the conclusion that the daily excretion of noradrenaline in such patients is not generally greater than normal, although in about 16 per cent of the cases the excretion was slightly greater than in subjects with normal blood pressures.

When noradrenaline is administered by slow intravenous infusion, it is found that a small part (0.3—4.0 %) of the infused amount is regularly excreted in the urine (GOLDENBERG 1951, v. EULER and LUFT 1951, PEKKARINEN and PITKÄNEN

1955 b, Elmadjian et al. 1956 b). A similar proportion (0.4—1.7 %) of adrenaline administered by infusion is also excreted in the urine (v. Euler, Luft and Sundin 1954). Evidently also an augmented endogenous secretion leads to an increased excretion in the urine.

The noradrenaline in the urine is considered to originate primarily in the sympathetic nervous system (Goldenberg and Rapport 1951). According to their observations, sympathectomy greatly decreases the noradrenaline content of the urine. Although the suprarenal medulla contains in addition to adrenaline 15 per cent noradrenaline, its removal does not significantly affect the noradrenaline excretion, but the amount of adrenaline in the urine is decreased by 80 per cent according to v. Euler, Frankson and Hellstöm (1954 a). Elmadjian et al. (1956 b) were unable to detect any trace of adrenaline in the urine following adrenalectomy. Also the fact that insulin hypoglycemia, which increases the adrenaline in the blood (Holzbauer and Vogt 1954), raises the adrenaline content of the urine in man (v. Euler and Luft 1952) and in animals (Pitkänen 1956) and at the same time decreases the adrenaline content of the suprarenal medulla, points strongly in favour of the assumption that the adrenaline in the urine primarily originates in the suprarenal gland. Furthermore, after adrenalectomy insulin causes no increase in the urinary adrenaline excretion in rats (Pitkänen 1956).

III

THE PRESENT INVESTIGATION.

DETERMINATION OF NORADRENALINE AND ADRENALINE IN URINE.

When large doses of sympathomimetic amines are administered orally, they are excreted in the urine mainly in the conjugated form (Richter 1940, Beyer and Shapiro 1945). The endogenous sympathomimetic amines, noradrenaline and adrenaline, are also excreted partly in conjugated form in the urine.

In many studies these amines have been liberated from their conjugates by acid hydrolysis. Beyer and Shapiro (1945) hydrolysed the urine on a boiling water bath for 30 minutes after adding 3 ml of concentrated sulphuric acid to a 40-ml sample. Holtz et al. (1947) heated the sample 20 minutes at 100° C, but they did not mention the pH of the mixture. v. Euler and Hellner (1951) hydrolysed the urine samples at a pH of 1—2 for 20 minutes at 100° C. These same conditions were used also by Burn (1953). Raab and Gigee (1954) added 0.2 ml of concentrated sulphuric acid to a 10-ml sample and heated the mixture 15 minutes. Nutzum and Bischoff (1953) added the same amount of acid to a 12.5-ml sample. Goldenberg et al. (1954) acidified the urine to pH 1—2 with hydrochloric acid and heated the sample 15 minutes on the boiling water bath. Elmadjian et al. (1956 a) concluded that the highest yield of noradrenaline is obtained when the sample is boiled at pH 1.5 for 15 minutes. These latter authors employed also enzymatic hydrolysis to decompose the glucuronide conjugates.

v. Euler and Orwén (1955) studied the effect of pH on the degree of hydrolysis and found that the amount of noradrenaline and adrenaline liberated increases with decreasing pH, the highest yield being obtained after hydrolysis at pH 0. They added 11 ml of 10 N HCI to 100 ml of urine and kept the sample 10 minutes at 100° C. The increase of activity was usually 1.5- to 3-fold, but during the hydrolysis the laevorotatory noradrenaline and adrenaline were transformed into the dl-forms, which have only half the activity of the l-forms. The values obtained in biological determinations after the hydrolysis had therefore to be doubled.

In the present investigation the object was avoid the changes caused by acid hydrolysis, and hence the extractions were made on unhydrolysed urine. *Consequently only the amounts of free biologically active noradrenaline and adrenaline were determined. All values for noradrenaline and adrenaline given in this publication refer to the l-forms.*

A. PREPARATION OF URINE FOR ASSAY.

1. Adsorption.

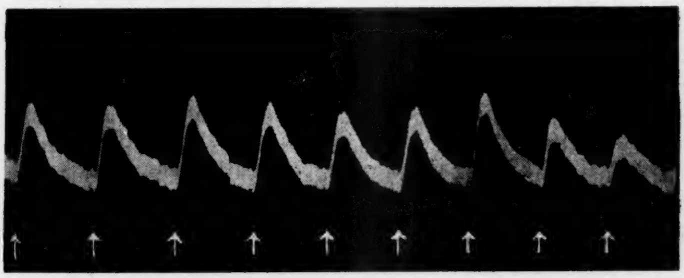
Since the concentrations of noradrenaline and adrenaline in urine are relatively low and since urine contains substances that may interfere with the analysis, it is necessary to perform an extraction of the urine before the assay.

Shaw published in 1938 a method in which adrenaline is adsorbed on aluminium hydroxide at pH 8.5. Holtz et al. (1947) purified their urine samples with lead acetate before adsorbing the sympathomimetic agents on aluminium hydroxide at pH 8. v. Euler (1948 b) showed that when the aluminium hydroxide is allowed to form from aluminium sulphate and sodium hydroxide in the sample solution, a smaller amount of aluminium hydroxide is sufficient to effect a complete adsorption already at pH 7.6. He and many other workers have employed this method in numerous studies on the noradrenaline and adrenaline contents of the urine. Pekkarinen (1948) dialysed adrenaline from blood through a cellophane membrane using aluminium hydroxide as adsorbent and found the optimal pH to be 8.5.

Lund (1949, 1950) used an aluminium oxide column for the adsorption of noradrenaline and adrenaline from the blood. Pekkarinen and Pitkänen (1955 a) employed aluminium oxide as adsorbent also prior to the chemical determination of noradrenaline in urine. It was also found possible to use the same adsorbent prior to the biological determination of noradrenaline and adrenaline in urine (Pekkarinen and Pitkänen 1954, Kärki 1954, v. Euler and Orwén 1955).

Since aluminium oxide has proved to be a more convenient adsorbent than the hydroxide, aluminium oxide was also employed for this purpose in the present study. The amount added per 25 ml of urine was one gram; this amount has previously been found sufficient for urine analyses (Pekkarinen and Pitkänen 1955 a). v. Euler and Orwén (1955) have reported that 2.5—3 grams of aluminium oxide per 100 ml of urine is a suitable ratio.

Prior to the adsorption, the pH of the urine sample was increased to 8.5 with 2 N sodium hydroxide using a potentiometer. Several samples were treated with aluminium oxide at the same time in an electrically driven stirrer system (for 4—6 samples) or in specially designed rocking apparatus (for 8—10 samples) (Pekkarinen and Pitkänen 1954). The adsorption of noradrenaline and adrenaline on aluminium oxide takes place rapidly in alkaline medium as seen in Fig. 1. Maximum adsorption of noradrenaline is obtained already after a mixing period of 45 seconds.



0.08 N   5 min.   0.1 N   1½ min.   0.08 N   ¾ min.   0.1 N   ¼ min.   0.05 N

Fig. 1. The effect of mixing time on the adsorption of noradrenaline from urine by aluminium oxide. The times of adsorption in minutes and the control amounts of noradrenaline (N) in micrograms are given under the arrows. Tested on cat's blood pressure.

Noradrenaline and especially adrenaline are known to oxidize rapidly to biologically inactive compounds in alkaline medium, but, on the other hand, the adsorption is most effective at pH 8—8.5 (Pekkarinen 1948, Lund 1950). When serial urine samples were treated, the mixing, centrifugation and washing took 25—30 minutes and samples were at pH 8.5 during this time. To determine whether inactivation of noradrenaline occurred during this period,

an experiment was performed in which the noradrenaline and adrenaline contents of urine samples left to stand at pH 8.5 and room temperature were compared with the contents of identical samples which were immediately subjected to aluminium oxide adsorption after increasing the pH to 8.5.

For this experiment urine was obtained from two persons (A and B). Ten 25-ml samples were measured from each urine and their pH values were increased to 8.5. Six samples from each group were extracted immediately and four samples after they had stood two hours at room temperature. The noradrenaline and adrenaline contents in the samples were determined biologically using the same test objects. The results in Table 1 show that when compared with the values for the samples which were immediately extracted, about 27—28 per cent of the noradrenaline and about 32—33 per cent of the adrenaline were inactivated during the 2-hour standing period. Since only about 30 per cent of the noradrenaline and adrenaline is inactivated during a period from 4 to 5 times the extraction period, the losses due to a high pH when the extraction is performed in the normal manner are relatively slight; this extraction operation must however be performed as rapidly as possible. In the present study, the samples remained at a high pH no longer than 25 minutes. MANN (1953) has stated that urine contains agents that inhibit oxidation; according to him the phosphates and vitamin C normally present in urine function as antioxidants.

The aluminium oxide was separated from the solution by centrifuging. A period of 3—5 minutes at 1500 r.p.m. was found adequate.

Table 1.

The inactivation of noradrenaline and adrenaline in urine at pH 8.5 and room temperature. The means I relate to six immediately extracted urine samples and the means II to four samples stored 2 hours at pH 8.5.

Urine	Noradrenaline μg/25 ml I	Noradrenaline μg/25 ml II	Decrease in per cent	Adrenaline μg/25 ml I	Adrenaline μg/25 ml II	Decrease in per cent
Urine A	0.52	0.38	27	0.09	0:06	33
Urine B	0.92	0.66	28	0.19	0.13	32

2. Elution.

Various methods have been employed to remove noradrenaline and adrenaline from adsorbents. HOLTZ, CREDNER and KRONEBERG (1947) used a primary sodium phosphate solution. v. EULER (1948 b) dissolved the aluminium hydroxide gel in sulphuric acid. The salts formed he precipitated with acetone-ethanol. After adjusting the clear solution to pH 3—4, he evaporated it under suction to a small volume. In their chemical determination of noradrenaline, PEKKARINEN and PITKÄNEN (1955 a) employed 1 N oxalic acid for the elution. PEKKARINEN and PITKÄNEN (1954) proposed the use of trichloracetic acid for the elution and its removal by ether extraction when the noradrenaline and adrenaline contents are high.

The elution and the evaporation in v. EULER's original method were time-consuming when series of 8—10 samples were treated, and therefore in the present study dilute (1 N) sulphuric acid was employed as eluant (cf. v. EULER and ORWÉN 1955, PITKÄNEN 1956).

Since the buffer capacity of the sediment varies, it was necessary to employ an excess of the sulphuric acid in the elution and usually the final pH of the sample was 1—1.5. The excess acid was neutralized with dilute sodium hydroxide to give a pH of 3.5—4; care was taken not to add too much alkali since adsorption begins when the pH rises to 5. The aluminium oxide was separated by effective centrifugation and the clear eluate was removed. The elution was repeated using distilled water. Owing to the sulphuric acid adsorbed by the aluminium oxide, the pH of the latter remained near 4; this was established with indicator paper.

3. Experimental procedure.

A twenty-five ml sample was taken for the extraction from each urine under study. Usually from 8 to 10 samples were treated in parallel. The pH values were adjusted to 8.5 with 2 N sodium hydroxide using a pH meter (Radiometer) and an electrically driven stirrer.

After the sample was transferred to a 30-ml centrifuge tube, one gram of aluminium oxide (Al_2O_3 for chromatographic adsorption analysis acc. to Brockman, from E. Merck AG, Darmstadt) was added to the sample, the mixture was shaken vigorously for 5 minutes. The sedimentation was effected by rapid centrifugation. The precipitate was washed twice with distilled water (25 ml), centrifuging after each washing.

For the elution 1 ml of 1 N sulphuric acid was added to each tube and the contents thoroughly mixed. After the mixing the pH of the samples was increased to 3.5—4 with 0.5 N sodium hydroxide using indicator paper. Following centrifugation at 2500 r.p.m., the clear solution was transferred to a graduated tube (eluate I). The second elution was performed by adding distilled water, 0.7—0.9 ml, so that the volumes of eluate I and the water together amounted to 2.5 ml. After the mixing and centrifuging this eluate (II) was added carefully to the eluate I in the graduated tube.

The total volume of eluate, 2.5 ml, contains the noradrenaline and adrenaline extracted from the 25-ml sample of urine originally taken. Usually this concentration ratio (1: 10) is suitable, but if the noradrenaline and adrenaline contents of the urine are lower than normal, as is frequently the case for urine excreted at night, it is necessary to concentrate to a smaller volume (e.g. 1: 20). The volume of urine taken was then 100 ml, but the procedure was otherwise practically the same.

The samples of pH 4 obtained in this way were very stable and could be used as such for bioassay on the cat's blood pressure and after neutralization for the estimation of their action on the hen's rectal caecum.

A part, about 50, of the 24-hour urine samples were extracted by a modification of the method described by v. EULER and HELLNER (1951). Instead of aluminium hydroxide, aluminium oxide was used for the adsorption. The oxide was eluted with acidified alcohol, and the eluate desalted with acetone-ethanol and evaporated under diminished pressure to a small volume.

The assays performed with these extracts gave results identical with those obtained with the previous procedure when the tests were made on the cat's blood pressure, but in the test on the hen's caecum, divergent results were frequently obtained. This may have been due to incomplete removal of the organic solvents and their variable action on the hen's caecum.

B. ESTIMATION OF NORADRENALINE AND ADRENALINE.

The observation that noradrenaline and adrenaline both are present in variable amounts in the organism has forced investigators to search for methods by which these amines can be estimated separately in their mixtures. Both chemical and biological methods have been introduced for the differentiation of noradrenaline and adrenaline in the same sample.

1. Chemical methods.

The chemical methods involve the transformation of noradrenaline and adrenaline into colored or fluorescent compounds. By means of various oxidants, it is possible to change noradrenaline and adrenaline into noradrenochrome and adrenochrome, which can be estimated colorimetrically. v. Euler and Hamberg (1949, 1950) used iodine as oxidizing agent, the differentiation being based on the different rates of oxidation of the amines at pH 4. This method is, however, too insensitive for samples such as urine which contain only small amounts of noradrenaline and adrenaline.

A considerably more sensitive chemical method is based on the measurement of the fluorescence (Loew 1918) of derivatives of the amines. This method was initially applied only to the estimation of adrenaline (Gaddum and Schild 1934, Kalaja and Savolainen 1941). The method was developed further by Pekkarinen (1948) who determined the adrenaline content of blood by means of dialysis and adsorption on aluminium hydroxide. Lund (1950) employed oxidation with manganese dioxide, Weil-Malherbe and Bone (1953) the condensation reaction with ethylenediamine, and v. Euler and Floding (1955) oxidation with potassium ferricyanide, to transform noradrenaline and adrenaline in biological samples into fluorescent derivatives.

Chemical fluorimetric methods have been employed for the estimation of noradrenaline and adrenaline in urine by Pekkarinen and Pitkänen (1955 a, b), Lund (1952), Raab and Gigee (1954), v. Euler and Floding (1955), and Pitkänen (1956).

2. Biological methods.

The biological methods for the estimation of noradrenaline and adrenaline in mixtures involve the study of the effect of the sample on two or more biological phenomena or organs on which the activities of the two amines differ greatly in magnitude. Since both noradrenaline and adrenaline produce a rise in blood pressure, an estimate of their combined effect may be obtained by studying the effect of the sample on the blood pressure of a spinal cat or anesthetized cat, rabbit or rat. By administering adrenolytic drugs such as ergotamine to the test animal and repeating the injection of the sample, it is possible to evaluate the amount of adrenaline from the suppression of the reaction (HOLTZ, CREDNER, and KRONEBERG 1947, HAMILTON et al. 1953). The ratio of the response of the cat blood pressure to that of the nictitating membrane gives an estimate of the adrenaline content of the sample (BURN, HUTCHEON and PARKER 1950, SHEPHERD and WEST 1951 b, WEST 1951). BÜLBRING and BURN (1949 a) used the different sensitivity of the normal and denervated nictitating membrane of the cat to noradrenaline for differentiating between the latter and adrenaline. SCHÜMANN (1949 a, b) determined the ratio of noradrenaline and adrenaline in suprarenal gland extracts from the difference in the effects of noradrenaline and adrenaline on the blood sugar and blood pressure.

GADDUM and LEMBECK (1949) studied the action of noradrenaline and adrenaline on the rat's uterus and colon stimulated with carbachol. Both amines can be differentiated accurately by this method, since the rat uterus is 75—350 times more sensitive to adrenaline than to noradrenaline, whereas, on the other hand, the colon is more sensitive to noradrenaline than to adrenaline (GADDUM 1950). This method has been widely employed in studies requiring a high sensitivity (e.g. GADDUM, PEART and VOGT 1949, PEART 1949, MANN and WEST 1950, BÜLBRING and BURN 1949 b).

In addition to having a sufficient sensitivity, the biological test preparation used for the determination of noradrenaline and adrenaline must not react to other biologically active compounds such as histamine, acetylcholine, 5-hydroxytryptamine possibly present in samples of biological origin.

The method proposed by v. EULER (1948 b) which measures the action of adrenaline and noradrenaline on the cat's blood pressure and on the hen's rectal caecum (BARSOUM and GADDUM 1935) will be described below in the form that it has been employed in the present investigation.

Blood pressure response in the cat and rat. The weights of the cats employed in the present study varied from 1.5 to 5 kg. Before the tests the cats fasted 12—18 hours, but had water *ad libitum.* Under ether anesthesia a cannula was fastened to the femoral vein and 50—60 mg of Chloralose was injected per kg of body weight. The trachea was cannulated and artificial respiration was given with a volume of 35—50 ml using a Starling Ideal pump. For recording the blood pressure, a mercury manometer was connected to the carotid artery by means of a glass cannula which had a inner diameter of 1—1.5 mm. Heparin was used to prevent clotting.

To stabilize the blood pressure and to sensitize the blood pressure responses, 2 mg/kg of atropine were administered subcutaneously, 8 mg/kg of cocaine intramuscularly and 0.1 mg/kg of ergotamine intramuscularly. The latter agent selectively inhibits the pressor receptor reflexes (v. EULER and SCHMITERLÖW 1944) and in small doses increases the action of sympathomimetic drugs (JANG 1941). Antihistaminics were used to avoid possible histamine activity, but also because they potentiate the action of noradrenaline and adrenaline on the blood pressure (PAASONEN 1953). When mepyramine maleate (Anthisan) was used, the intramuscular dose varied from 3 to 5 mg per kilogram of body weight, and when chlorprophenpyridamine maleate (Chlor-trimeton) was used, from 1 to 2 mg/kg. Larger doses of these substances than these increase the duration of the blood pressure response.

In some cases, the cat's blood pressure was high at the start of the experiment and the response to adrenaline was often a decrease. When the blood pressure did not change during one or two hours, it was found advantageous to inject a small quantity (2—5 mg/kg) of hexamethonium intramuscularly or intravenously in small doses; the sensitivity to hexamethonium varies in different cats. In this way the blood pressure response usually became so sensitive that a 0.1-μg dose of noradrenaline caused a pressure increase of 30—40 mm Hg (BARTONELLI et al. 1954). — The sensitivity of the blood pressure reaction is very important when small amounts of nor-

adrenaline in the urine eluate are being estimated. — The dose ratio of noradrenaline and adrenaline varied for the same response from 0.1 to 0.5. The samples under study were injected into the femoral vein in 0.25—0.5-ml volumes and after each injection the cannula was rinsed with 1—1.5 ml of physiological saline. The tests were performed at intervals of 3—5 minutes depending on the time required for the blood pressure to become normal again. After each injection of a urine eluate of unknown activity, a known amount of standard solution was administered and the response recorded.

When the tests were made on the rat's blood pressure, the mercury manometer used differed from the ordinary type. The area of the mercury surface in the branch on which the pressure acted was about 50 times greater than in the other branch which gave the readings (CRAWFORD and OUTSCHOORN 1951). With this manometer the pressure reading variations are almost twice those obtained with a manometer in which both branches are equal in diameter.

The rats were anesthetized by administering 60—80 mg/kg of chloralose and 20 mg/kg of pentobarbital intraperitoneally. Both vagi were severed and the trachea was cannulated for artificial respiration. A polythene cannula was inserted in the femoral vein for the injection. A glass cannula fastened to the common carotid artery was connected to the manometer by polythene tubing 3 mm in diameter. For the stabilization of the blood pressure and to increase the sensitivity, 10 mg/kg of hexamethonium were administered intramuscularly. The injections were given in 0.05—0.15-ml volumes and the cannula was rinsed with a small volume of physiological saline sufficient to give the same volume of injected liquid. The ratio of the effect of noradrenaline to adrenaline on the blood pressure was not usually as favorable as in the tests made on the cat's blood pressure, but varied from 0.3 to 1.0.

Tests conducted in parallel revealed that fully comparable results were obtained when the effects on the cat and rat blood pressures were measured.

Hen's rectal caecum. The hens were killed by decapitation. The abdomen was opened and a piece 4—5 cm in length of the proximal portion of the caecum required in the test was suspended in a 25-ml bath at 38° C. Only freshly prepared Tyrode solution was used

as the bath liquid. The standard stock solutions were prepared as recommended by Hökfelt (1951).

Stock solution I.

NaCl	200.0 g
KCl	2.5 g
$CaCl_2$ (dry)	5.0 g
$MgCl_2$, 6 H_2O	5.0 g

Dissolved in distilled water ad 1000 ml.

Stock solution II.

$NaHCO_3$	50.0 g
NaH_2PO_4 , H_2O	2.5 g

Dissolved in distilled water ad 1000 ml.

Eighty ml of solution I and forty ml of solution II were separately diluted to 1000 ml with distilled water. The two solutions were then mixed and 1 g of glucose was added per litre.

To guarantee an adequate sensitivity, the caecum was carefully balanced. The movements were recorded with a frontal level on a smoked drum with a magnification of 4—6. During the test the degree of stretch and a relatively high flow of oxygen were held constant. An adequate relaxation was usually effected with 0.15—0.6 μg of noradrenaline and 0.005—0.02 μg of adrenaline; the activity ratio of adrenaline to noradrenaline varied from 10 : 1 to 40 : 1.

The samples under test were neutralized to 7.0—7.4 with a $NaHCO_3$—NaH_2PO_4 solution immediately before they were added to the bath. The volumes of the urine extracts required in the tests varied from 0.005 to 0.5 ml. The samples were added to the bath at intervals of about 3—5 minutes and they were allowed to exert their action from 45—60 seconds depending on the sensitivity of the caecum (Fig. 2).

When the effect of the sample on both the blood pressure and on the hen's rectal caecum had been evaluated, the noradrenaline and adrenaline contents were computed from equations presented by v. Euler (1948).

A = μg of l-noradrenaline equivalents per 25 ml of urine on hen's rectal caecum.

a = μg of l-noradrenaline equivalents per 25 ml urine on cat's blood pressure.

Q = activity ratio l-adrenaline/l-noradrenaline on hen's rectal caecum.

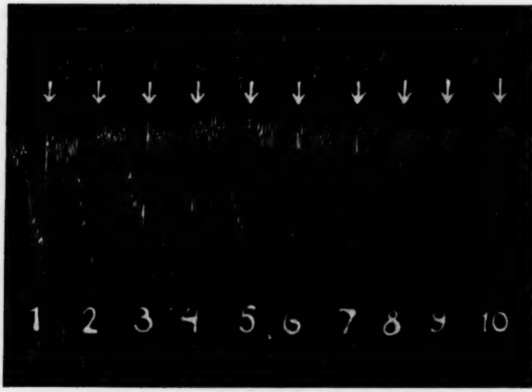


Fig. 2. Assay on hen's rectal caecum. Bath volume 25 ml. Washing and 5 minutes' recovery opposite each arrow.

1. 0.3 microgram of noradrenaline
2. 0.25 ml of urine extract no. 363 (urine volume 2.5 ml)
3. 0.125 ml of urine extract no. 363 (urine volume 1.25 ml)
4. 0.2 microgram of noradrenaline
5. 0.013 microgram of adrenaline
6. 0.35 microgram of noradrenaline
7. 0.25 ml of urine extract no. 365 (urine volume 2.5 ml)
8. 0.38 microgram of noradrenaline
9. 0.01 microgram of adrenaline

q = activity ratio l-adrenaline/l-noradrenaline on blood pressure.

$$x = \text{l-adrenaline in } \mu g \text{ per 25 ml of urine} = \frac{A - a}{Q - q}.$$

$$y = \text{l-noradrenaline in } \mu g \text{ per 25 ml of urine} = A - xQ.$$

C. STATISTICAL TREATMENT.

In the statistical analysis of the results, the usual methods have been applied (see e.g. FISHER 1950). In addition to the mean ($\bar{x}$), the standard deviation (S.D.) and standard error of the mean (S.E.) have been calculated from the formulas

(1) $S.D. = \sqrt{\left[\Sigma x^2 - (\Sigma x)^2 / n\right] / (n-1)}$

and

(2) $S.E. = S.D./\sqrt{n}$,

where x stands for the individual observation and n is their number. In analyses concerned with an observed increase or decrease in the variable under investigation, x stands for the observed change in each individual case. $\bar{x}$ then represents the average change, the statistical significance of which can be assessed by Student's t-test:

(3) $t = \bar{x}/S.E.$

By consulting appropriate statistical tables (e.g. FISHER and YATES 1953), a probability P can be determined which indicates the likelihood that the observed average change is a result of chance variations only. If this probability is equal to or smaller than 0.05 (5 per cent), the change is judged to be statistically significant.

In some instances, more than two averages are to be compared. In such situations the analysis of variance has been applied. In essence, this type of analysis is based on the same principles as the t-test in its simplest form, but is, of course, more complicated. The results of this analysis also can be given in the form of probability values P which indicate the probability that the observed differences are due to chance variations only.

Sometimes a simple statistical test, known as the sign test, has been applied. For instance, if a definite diurnal rhythm is apparent in the urine excretion, the difference between the amounts excreted during successive periods may sometimes be positive (increase, plus sign), sometimes negative (decrease, minus sign), the number of plus and minus signs being, on an average, equal. If, on the other hand, a marked difference exists between the number of plus and minus signs, this may provide statistically significant evidence for the existence of a definite rhythm. This evidence can again be evaluated in terms of the probability P that the observed sign distribution is due to random variations only.

In regression analysis it is assumed that the dependent variable y (adrenaline excretion, say) is, on average, a linear

function of the independent variable x (body weight, say), which can be expressed in the form of a regression equation.

(4) $y \simeq a + bx$.

The parameters a and b of the equation have been estimated by means of the method of least squares, and the significance of the relation has been tested by the analysis of variance. If the regression is found significant, the linear equation corresponds well to the observations, otherwise not.

D. INVESTIGATIONS PERTAINING TO THE TECHNIQUE.

Since the preparative treatment of urine deviates in some respects from the methods previously employed, a number of experiments were made to determine the effect of the difference in treatment.

Recovery. Urines from two human subjects (E. I. and N. K.) were divided into two series of nine 25-ml samples. 0.5—3.0 μg of noradrenaline and 0.1—1.0 μg of adrenaline were added to the first six samples of each series. Three samples from both series were retained as controls. From all samples of both urines noradrenaline and adrenaline were adsorbed on aluminium oxide and centrifuged, washed and eluted with sulphuric acid as described above. The extracts were tested biologically for their effect on the blood pressure of the same cat and on the same hen's caecum. The results are shown in Table 2.

The amounts of noradrenaline and adrenaline initially present in the urines were computed from the results obtained for the three control samples. For the urine E. I. the mean contents were 0.85 μg of noradrenaline and 0.21 μg of adrenaline per 25 ml, and for urine N. K. 0.5 μg and 0.12 μg, respectively. These initial values were subtracted from the values found for the samples to which noradrenaline and adrenaline had been added. The mean recovery from the urine E. I. was 72.3 per cent for noradrenaline and 72.8 per cent for adrenaline. The respective percentages for

Table 2.
Recovery of noradrenaline and adrenaline added to urine. Amounts in micrograms per 25 ml.

Sample No.	Noradrenaline initially	Added	Noradrenaline initially + added	Noradrenaline found	Noradrenaline recovered	Percentage recovery	Adrenaline initially	Added	Adrenaline initially + added	Adrenaline found	Adrenaline recovered	Percentage recovery	Urine
1	0.85	0.50	1.35	1.24	0.39	78.0	0.21	0.10	0.31	0.28	0.08	80.0	E. I.
2	0.85	1.0	1.85	1.64	0.79	79.0	0.21	0.20	0.41	0.36	0.15	75.0	,,
3	0.85	1.5	2.35	1.84	0.99	66.3	0.21	0.40	0.61	0.50	0.29	72.5	,,
4	0.85	2.0	2.85	2.20	1.35	67.5	0.21	0.60	0.81	0.61	0.40	66.7	,,
5	0.85	2.5	3.35	2.68	1.83	74.0	0.21	0.80	1.01	0.80	0.59	73.8	,,
6	0.85	3.0	3.85	2.92	2.00	69.0	0.21	1.00	1.21	0.90	0.69	69.0	,,
					Mean	72.3					Mean	72.8	
					S. D.	5.48					S. D.	4.68	
					S. E.	2.24					S. E.	1.91	
1	0.50	0.50	1.0	0.89	0.39	78.0	0.12	0.10	0.22	0.20	0.08	80.0	N. K.
2	0.50	1.00	1.50	1.27	0.77	77.0	0.12	0.20	0.32	0.25	0.13	65.0	,,
3	0.50	1.50	2.00	1.60	1.06	70.7	0.12	0.40	0.52	0.40	0.28	70.0	,,
4	0.50	2.00	2.50	1.92	1.42	71.0	0.12	0.60	0.72	0.55	0.43	71.7	,,
5	0.50	2.50	3.00	2.18	1.68	67.2	0.12	0.80	0.92	0.70	0.58	72.5	,,
6	0.50	3.00	3.50	2.67	2.17	72.3	0.12	1.00	1.12	0.85	0.73	73.0	,,
					Mean	72.7					Mean	72.0	
					S. D.	4.10					S. D.	4.87	
					S. E.	1.67					S. E.	1.98	

urine N. K. were 72.7 and 72.0. The recoveries varied in both urines from 66 to 80 per cent. The recoveries of added noradrenaline and adrenaline are seen to be practically equal, which indicates that the urines did not differ in this respect from each other.

The amounts of noradrenaline and adrenaline added to the urine were relatively small since it was intended that their contents were to remain within the range of normal variation in urine. Similar recoveries were also obtained in tests in which larger (up to tenfold) additions were made.

Error of method. In order to determine the reproducibility of the combined extraction and biological estimation procedure, i.e. to evaluate the methodical error, an experiment was performed in which replicate determinations, 15 in all, were made on two urines. The replicate samples from both urines were extracted in one group and tested on the same cat and the same hen's rectal caecum. The methodical error, expressed as the square root of the variance,[1] has been calculated separately for both urines. The results are shown in Table 3. The methodical error amounts to 8—10 per cent of the mean noradrenaline content and 6—8 per cent of the mean adrenaline content.

Stability of the extracts. Sometimes the urine extracts were stored for periods up to one week before the biological estimations were made. The extracts had pH values varying from 3.5 to 4 and were stored in closed tubes at $+2 - +4°$ C. To determine whether inactivation of noradrenaline and adrenaline or other changes occurred during storage, an experiment was performed in which extracts (the same extracts for which results are given in Table 3) were stored for 10 days and their noradrenaline and adrenaline contents were again estimated. The mean changes which the contents of these substances underwent appear from

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[1] For the determination of the error of method, the sum of squares $\Sigma\ (x-\bar{x})^2$ has been calculated for each urine in the usual manner and the two sums of squares have then been pooled and the total divided by the number of degrees of freedom. The square root of this variance gives the error of the method.

$$s = \sqrt{\left[\Sigma\Sigma(x-\bar{x}_i)^2\right] / \left[\Sigma(n_i-1)\right]}$$

Table 3.

The calculation of the error of method. Results for samples of urines from two persons (P.V. and N.K.).

Urine	Sample No.	Noradrenaline µg/25 ml	Adrenaline µg/25 ml	Total	Per cent Adrenaline
P. V.	1	0.93	0.27	1.20	22
	2	0.89	0.30	1.19	26
	3	0.92	0.28	1.20	23
	4	0.99	0.26	1.25	20
	5	1.00	0.30	1.30	23
	6	0.81	0.30	1.11	27
	7	0.82	0.30	1.12	27
	8	0.81	0.30	1.11	27
	9	0.96	0.28	1.24	23
Mean		0.903	0.288	1.191	24.2
Error of method		0.075	0.016	0.067	2.6
Per cent		8	6	6	11
N. K.	1	0.58	0.10	0.68	15
	2	0.58	0.10	0.68	15
	3	0.52	0.10	0.62	17
	4	0.45	0.09	0.54	17
	5	0.48	0.10	0.58	16
	6	0.54	0.08	0.62	13
Mean		0.525	0.095	0.620	15.5
Error of method		0.053	0.008	0.055	1.5
Per cent		10	8	9	10

Table 4. The levels of significance of the differences were estimated with the aid of Student's t-test. No significant changes occurred in the noradrenaline and adrenaline contents during the ten-day period. The same applies to the ratio of adrenaline content to the total content of noradrenaline and adrenaline.

Effect of temperature and pH on the stability of noradrenaline in urine. Noradrenaline and adrenaline are readily oxidized and lose their biological activity at high pH values. A high temperature accelerates the inactivation. Although attempts were made to cool the urine samples as soon as possible after they were collected,

Table 4.

Variation of noradrenaline and adrenaline contents of urine extracts stored for 10 days at $+2°$—$+4°$ C. The results obtained when the extractions were performed immediately are given in Table 3 (urine P.V.). The numbers of the samples are the same as in Table 3.

Urine	Sample No.	Change in noradrenaline content	Change in adrenaline content	Change in total noradr. and adrenal. content	Change in percentage of adrenaline
P.V.	1	—0.03	+ 0.06	+ 0.03	+ 5
	2	—0.11	—	—0.11	+ 2
	3	—0.12	+ 0.02	—0.10	+ 4
	4	—0.06	+ 0.10	+ 0.04	+ 7
	5	—0.12	—	—0.12	+ 2
	6	—0.01	—0.01	—0.02	—1
	7	+ 0.18	+ 0.01	+ 0.19	—3
	8	—0.01	—	—0.01	—
	9	—0.01	+ 0.01	—	—
Mean change $\bar{d}$ ..		—0.032	0.021	—0.011	1.8
S. E. of mean change		0.031	0.012	0.032	1.1
$t = \bar{d}$/S. E.		1.03	1.75	0.34	1.64
D. F.		8	8	8	8
P		> 0.1	> 0.1	> 0.1	> 0.1

this could not always be done and some of the urine samples remained several hours after their excretion at room temperature. For this reason sulphuric acid was initially added to the containers reserved for urine to lower the pH of the urine to 4—5.

The following experiments were performed to assess the effect of temperature and pH on the noradrenaline content of urine. One microgram/ml of noradrenaline was added to a urine sample. The sample was then divided into four subsamples, of which two were adjusted to pH 4 and two to pH 6.5. One of each pair of subsamples was stored at $+4°$ C for one week. The other two samples were first stored 2 days at room temperature ($+20$—$+23°$ C) and then for five days with the other samples at $+4°$ C. Extracts were prepared from all four urine samples for the estimation of their noradrenaline contents from the effect on the cat's blood pressure.

It was established (Fig. 3) that the noradrenaline contents of the samples remained constant when the latter were stored one week at +4° C irrespective of whether the pH was 4 or 6.5. A storage at room temperature for two days was, however, sufficient to reduce the activity of noradrenaline by 70—80 per cent when the pH of the sample was 6.5, whereas no change in activity was noted when the pH of the sample was 4 during the storage period.

It was found that the pH of the sample which was initially 6.5 rose to 8 during the 2-day storage at room temperature. This was evidently due to the production of ammonia by bacteria.

Fig. 3. Stability of noradrenaline added to urine. One μg of noradrenaline added per ml of urine. Equal volumes of extracts of samples of one urine stored under different conditions have been tested. The control amounts of noradrenaline (N) are given in micrograms. Cat's blood pressures in mm of mercury.

A Urine sample of pH 4 stored 7 days at + 4° C.

B Urine sample of pH 6.5 stored 7 days at + 4° C.

C Urine sample of pH 4 stored 2 days at + 23—+ 25° C and 5 days at + 4° C.

D Urine sample of pH 6.5 stored 2 days at + 23—+ 25° C and 5 days at + 4° C.

THE NORMAL EXCRETION OF NORADRENALINE AND ADRENALINE IN URINE.

A. THE TWENTY-FOUR-HOUR EXCRETION.

1. Material.

The collection of urine excreted during 24 hours was usually begun in the morning. The bladder was emptied immediately on awakening and all urine excreted during the next 24 hours was combined.

Since the inactivation of noradrenaline and adrenaline takes place most slowly at low pH values, sulphuric acid was added beforehand to the containers into which the urine was collected. The usual addition of 3—5 ml of 2 N sulphuric acid reduced the pH of the urine to 4—5. The extraction was in most cases performed immediately after the 24-hour urine had been collected, but if an interval of one or more days intervened, the pH of the sample was adjusted to 4 and the sample transferred to a refrigerator.

The concentrations of adrenaline and noradrenaline in the urine of children were frequently low, since the volume of urine excreted daily by children a few years old was often as high as 1000 ml. In such cases the extraction was performed on 100-ml instead of 25-ml samples and the final volumes of the extracts were then 5 ml.

The adrenaline was not always evaluated for all urine samples; in these cases the noradrenaline content was expressed in terms of noradrenaline equivalents on the basis of the effect on the cat's blood pressure. The error that results from the adrenaline present in normal urine is small since the effect of adrenaline on the blood pressure is only about one-fourth to one-fifth of that of noradrenaline and the content of the former is on the average only one-fifth of the noradrenaline content.

In most cases, only one 24-hour urine from each subject was examined, but for a number of subjects the daily secretion of noradrenaline and adrenaline was followed over a period of several days.

The subjects comprised 175 male and 116 female persons. The number of 24-hour urine samples examined for noradrenaline was 240 and for adrenaline 356. The ages of both men and women varied from 1.5 to 96 years. When evaluating the results, the subjects were divided into five age groups, from 1.5 to 6 years, from 7 to 16 years, from 17 to 29 years, from 30 to 59 years, and over 60 years. The subjects for the study were chosen so that all persons in the same group were employed in the same work or in similar work during the time the urine samples were collected. Persons engaged in heavy manual labour were not included in these groups. The occupations or activities of the persons belonging to the different age groups will be given when the results are discussed.

2. Results.

The variation of the excretion with age.

a. Children from 1.5 to 6 years of age. The children of this group were from municipal or private children's homes. All were in good health. During the collection of the urine samples, no restrictions were placed on their freedom, foods or other daily habits. The nurses of the institutions took care of the collection of samples. Only those subjects were included in the study from whom the whole 24-hour urine excretion was collected.

The total number of the children in this group was 42. The average age of the boys was 3.8 years, and their average weight 16.2 kg. The noradrenaline content was determined on 41 24-hour urine samples and the adrenaline content on 26 24-hour urine samples from this group of boys. The results are shown in Table 5. Since the members of this group varied greatly in weight and activity, it is natural that also the results for this group show marked variation. The noradrenaline excreted varied from 3.5 to 13.3 μg with an average excretion of 6.4 (S. E. 0.44) μg/24 hrs. The excretion of adrenaline varied from 0.7 to 2.2 μg und averaged 1.3 (S. E. 0.12) μg/24 hrs. The mean ratio of adrenaline to total adrenaline and noradrenaline was 19.1 %.

The average age of the girls was 3.2 years and the average weight 14.4 kg. The noradrenaline was determined in the 24-hour

Table 5.

Daily excretion of noradrenaline and adrenaline by boys from 1.5 to 6 years old.

Subject No.	Age	Weight kg	Urine volume ml	Noradrenaline μg	Adrenaline μg	Per cent adrenaline
334	1.5	12	550	4.3		
31	2	13	390	5.3		
271	2	15	475	4.3	1.1	20.4
336	2	14	430	4.0	0.9	18.4
61	3	15	355	8.2		
307	3	16	1040	7.7	2.0	20.6
312	3	16	1040	6.0	1.2	16.7
337	3	13	505	5.5	1.2	17.9
338	3	16	755	4.1	2.2	34.9
30	3	14	390	5.2		
391	3	17	322	6.2	1.6	20.5 [1]
339	3.5	12	510	5.1	0.8	13.6
331	3.5	14	580	4.1	1.1	21.2
56	4	15	360	11.3		
58	4	17	470	11.0		
59	4	14	340	6.6		
62	4	14	170	3.5		
63	4	14	200	6.0		
64	4	18	200	5.3		
273	4	18	500	7.3	1.7	18.9
304	4	16	500	4.3	1.0	18.9
306	4	18	1000	7.5	1.4	16.9
311	4	16	1000	6.7	1.0	13.0
60	5	19	400	9.3		
269	5	20	400	6.0	1.7	22.1
272	5	23	650	7.1	2.2	23.6
274	5	16	675	6.8		
55	6	22	740	13.3		
305	6	21	850	4.6	1.0	17.8
308	6	19	725	6.5	0.7	9.0
Mean	3.8	16.2	551	6.43	1.34	19.1
S. D.			249	2.39	0.48	5.4
S. E.			45	0.44	0.12	1.3

[1] Mean for 12 days.

Table 6.

Daily excretion of noradrenaline and adrenaline by girls from 1.5 to 6 years old.

Subject No.	Age	Weight kg	Urine volume ml	Noradrenaline µg	Adrenaline µg	Per cent adrenaline
75	1.5	11	210	3.5		
268	2	11	245	2.5		
332	2	11	300	1.5	0.6	28.6
333	2	13	350	3.5	0.8	18.6
341	2.5	12	800	6.6	1.7	20.5
54	3	14	155	3.4		
309	3	16	1050	3.6	0.5	12.2
340	3	16	645	3.9	1.3	25.0
275	4	15	275	5.1	1.1	17.7
276	4	18	325	6.2	1.7	21.5
310	5	16	985	9.0	2.4	21.1
303	6	20	600	4.8	1.2	20.0
Mean	3.2	14.4	495	4.47	1.26	20.6
S. D.			312	2.03	0.61	4.6
S. E.			90	0.59	0.20	1.5

urines of 12 girls and adrenaline in the 24-hour urines of 9 girls (Table 6). The range of the noradrenaline excretion was 1.5 to 9.0 µg with a mean excretion of 4.5 (S. E. 0.59) µg/24 hrs. The adrenaline excreted averaged 1.3 (S.E. 0.20) µg/24 hrs. and amounted to 20.6 per cent of total noradrenaline and adrenaline.

b. Children from 7 to 16 years. The members of this group were mostly from boarding schools. The collection of urine was performed during vacations under the supervision of their nurses. The number of subjects was 50.

The noradrenaline content was determined in 30 24-hour urine samples, and the adrenaline content in 19 24-hour samples collected from boys (Table 7). The average age of the boys was 10.8 years and the average weight 33.6 kg, which is approximately twice the average weight of the boys of the preceding group. The daily excretion of noradrenaline varied from 6.2 to 33.6 µg, with a mean of 16.0 (S. E. 1.41) µg/24 hrs., which is more than twice the mean value for the children of the preceding group. Also the adrenaline excretion was clearly higher in this group. The mean adrena-

Table 7.

Daily excretion of noradrenaline and adrenaline by boys from 7 to 16 years old.

Subject No.	Age	Weight kg	Urine volume ml	Noradrenaline µg	Adrenaline µg	Per cent adrenaline
97	7	24	860	6.2		
104	8	26	1160	10.1		
105	8	24	635	13.2		
114	8	24	1480	14.7	3.0	16.9
117	8	22	1560	17.6	3.3	15.7
96	9	25	980	7.0		
98	9	22	1200	10.8		
110	9	33	1500	10.5	2.3	18.0
112	9	29	1200	21.6	1.9	8.1
116	9	33	750	12.9	3.5	21.3
241	9	28	690	7.4	0.9	10.8
99	10	31	1050	12.6		
100	10	32	1930	11.1		
107	10	36	730	15.5		
236	10	20	610	10.9	3.4	23.8
113	10	38	1280	10.5	1.9	15.3
115	10	27	1375	18.0		
109	11	38	1125	8.1		
237	11	30	1040	11.6	1.3	10.1
106	12	36	1450	15.1		
238	12	36	1310	25.0	3.5	12.3
111	13	33	1370	10.1	2.9	22.3
227	13	42	1025	9.6	1.0	9.4
229	13	38	1440	19.1	3.5	15.5
232	13	35	1760	27.0	4.1	13.2
235	13	41	1140	25.3	2.2	8.0
231	14	48	1075	31.0	5.1	14.2
233	14	48	1730	28.3	3.9	12.1
228	15	50	1480	33.6	6.6	16.4
230	16	60	1100	25.8	2.8	9.8
Mean	10.8	33.6	1201	16.01	3.01	14.4
S. D.			338	7.71	1.40	4.7
S. E.			62	1.41	0.32	1.1

line excretion was 3.0 (S. E. 0.32) µg/24 hrs. and the extremes 0.9 µg and 6.6 µg per 24 hours. The percentage of adrenaline was 14.4 on the average.

Noradrenaline determinations were made on 36 24-hour urine samples and adrenaline determinations on 21 24-hour urine samples from girls of this group (Table 8). The average age of the girls was 10.1 years and the average weight 14.4 kg, which is slightly lower than the average weight of the boys of the same group. The excretion of noradrenaline was also slightly lower for the girls than that for the boys of this group and averaged 12.3 (S. E. 1.15) $\mu g/24$ hrs; the range was 6.1 to 23.0 $\mu g/24$ hrs. Although also the amount of adrenaline excreted by girls was

Table 8.

Daily excretion of noradrenaline and adrenaline by girls from 7 to 16 years old.

Subject No.	Age	Weight kg	Urine volume ml	Noradrenaline μg	Adrenaline μg	Per cent adrenaline
171	7	23	620	8.5		
173	7	24	500	10.0		
392	8	25	533	10.2	1.9	15.7 [1]
93	8	30	1200	7.2		
95	8	22	1550	10.9		
102	8	25	800	12.0		
103	8	23	1420	10.0		
174	9	30	550	14.0		
225	9	28	1140	6.1	1.7	21.8
393	9	30	597	15.1	2.1	12.2 [2]
394	10	33	538	17.4	2.0	10.3 [3]
170	11	28	1050	18.9		
226	11	35	1300	7.8	1.9	19.6
101	11	26	1655	16.5		
166	12	38	575	23.0		
239	12	38	1500	7.8	4.7	37.6
94	13	40	1700	10.2		
169	13	46	780	23.3		
234	13	36	1200	9.4	3.2	25.4
240	14	40	1610	7.7	1.6	17.2
Mean	10.1	31.0	1041	12.30	2.39	20.0
S. D.			488	5.05	1.08	8.6
S. E.			98	1.15	0.37	3.1

[1] 8 days. [2] 9 days. [3] 2 days.

smaller than that for the boys, the average excretion for the former being 2.4 (S. E. 0.37) $\mu g/24$ hrs., the ratio of adrenaline to total noradrenaline and adrenaline was higher for the girls (20 %) than for the boys (14.4 %).

In the groups of children from 1.5 to 16 years old, whose sizes and weights varied greatly, highly significant regressions were found between the amount of noradrenaline excreted during 24 hours and the body weight for both boys and girls.

	Number	Mean weight kg	Noradr. $\mu g/24$ hrs.	Regression coefficient	P
Boys	60	24.9	11.2	—2.2	< 0.0005
Girls	32	24.8	9.4	— 0.4	< 0.0005

c. *Age group from 17 to 29 years.* This group comprised 85 subjects. The men, 48 in number, were mostly recruits in active military service. The urine samples were collected during the usual programme of lectures, exercises, etc., but not during strenuous marches or competitions. The average age of the men was 23 years, and the average weight 72.4 kg.

The noradrenaline determinations were made on 69 24-hour urines and adrenaline determinations on 47 24-hour urines collected from the men. The daily excretions for these subjects are given in Table 9. The amount of noradrenaline excreted is seen to vary from 13.2 to 58.0 μg with a mean excretion of 25.7 (S. E. 1.42) $\mu g/24$ hrs. The mean excretion of adrenaline was 5.9 (S. E. 0.36) $\mu g/24$ hrs., the range being 2.9 to 12.0 $\mu g/24$ hrs. The ratio of adrenaline was 21.4 per cent.

The women of this age group were student nurses or laboratory workers. The urine was collected during the normal workday. The mean age of the women was 23 years, i.e. the same as for the men of this group. The women deviated clearly from the men in weighing 12 kg less on the average; the average weight for the former was 60.2 kg.

Noradrenaline was determined in 47 24-hour urine samples and adrenaline in 36 24-hour urine samples collected from the women. As could be expected in view of their lower weights, the amounts excreted were smaller for the women than for the men (Table 10). The noradrenaline excretion varied from 12.5 to 42.2 $\mu g/24$ hrs.,

Table 9.

Daily excretion of noradrenaline and adrenaline by men from 17 to 29 years old.

Subject No.	Age	Weight kg	Urine volume ml	Noradrenaline µg	Adrenaline µg	Per cent adrenaline
11	18	67	1250	16.0	9.6	37.5
80	18	68	1085	28.7		
14	19	70	1800	19.4	6.2	24.2
15	20	75	2250	32.1	4.1	11.3
18	20	75	2000	15.0	2.9	16.2
21	20	59	800	18.0	3.3	15.5
22	20	70	1670	41.5		
76	20	56	950	31.6		
78	20	70	1570	37.5		
1	20	77	990	25.2	5.6	18.2 [1]
2	20	83	1570	16.7	6.1	26.8 [2]
401	20	75	1175	58.0		
402	20	64	860	32.5		
403	20	66	760	33.4		
404	20	75	1155	38.5		
405	20	67	1210	47.0		
283	20	68	895	22.5	6.5	22.4 [3]
284	20	73	843	25.8	5.3	17.0 [4]
285	20	65	1270	33.1	6.7	16.8 [5]
287	20	71	840	31.2	5.1	14.0 [6]
9	21	76	1500	31.1	7.9	20.3
10	21	70	1500	23.4	4.5	16.1
12	21	75	2050	26.6		
286	21	70	1030	27.1	6.7	19.8 [7]
5	22	68	1853	19.9	6.5	24.6 [8]
380	22	74	1080	15.3	4.5	22.7
81	23	70	850	37.4		
4	23	73	965	13.6	4.6	25.3 [9]
322	23	69	820	23.8	5.1	17.6
353	23	69	1420	22.0	8.3	27.4
378	24	84	1525	35.0	7.3	17.3
381	24	73	1220	14.7	4.7	24.2
382	24	83	920	14.4	3.1	17.7
13	24	75	1450	15.6	6.6	29.7
16	25	75	1400	17.5	6.7	27.7
6	25	88	670	22.1		
19	26	77	850	19.2	4.8	20.0
77	26	69	1030	21.9		
395	26	90	670	22.7		
379	26	73	940	15.0	4.2	21.9
28	26	70	1200	13.2		
20	27	70	900	24.1	4.6	16.0
79	27	81	1150	37.3		
209	27	70	840	40.0	12.0	23.1
368	27	71	1050	16.0	4.4	21.6
181	28	75	620	22.0	8.7	28.3
374	29	75	1270	22.2	6.3	22.1
33	29	68	800	16.5		
Mean	23	72.4	1177	25.69	5.90	21.4
S. D.			387	9.89	1.98	5.5
S. E.			56	1.42	0.36	1.0

[1] 3 days. [2] 3 days. [3] 4 days. [4] 4 days. [5] 4 days. [6] 2 days. [7] 4 days. [8] 3 days. [9] 3 days.

with a mean of 22.9 (S. E. 1.14) μg/24 hrs. The adrenaline excretion varied within wider limits, from 0.6 to 7.9 μg, with a mean excretion of 4.2 (S. E. 0.38) μg/24 hrs. The percentage of adrenaline (15.3) was also lower than for the men. When the amounts of noradrenaline and adrenaline excreted daily by the persons of this group are calculated per kg of body weight, the differences between the men and women level off, as will be shown later.

It is interesting to note that as the weights of the subjects increased about twofold as compared with those of the preceding age group, the amounts of noradrenaline and adrenaline excreted also underwent an approximately proportionate increase.

d. Age group from 30 to 59 years. This group comprised 57 middle-aged persons.

The 36 men were engaged in office work or in other indoor occupations. Their average age was 45 years and average weight 74.4 kg.

Thirty-eight 24-hour urine samples collected from these men were examined for their noradrenaline content and 23 samples for their adrenaline content (Table 11). The mean noradrenaline excretion was found to be 25.2 (S. E. 1.63) μg/24 hrs., which is almost the same as the value obtained for the men from 17 to 29 years of age. The variation of noradrenaline excretion was rather large (12.0—49.4 μg/24 hrs.) The mean amount of adrenaline excreted daily by the men was 6.5 (S. E. 0.70) μg/24 hrs., and the range 2.1 to 12.6 μg/24 hrs. The ratio of adrenaline to the total noradrenaline and adrenaline was 20.3 per cent.

The women of this group performed various duties in the hospital. A part (8 cases) of these were convalescents in wards, who moved freely about and whose diseases were of minor nature. The average age of the women was 42 years and the average weight 71.2 kg, i.e. appreciably higher than for the women in the group from 17 to 29 years. The difference between the weights of men and women in this age group was smaller than in preceding age group.

Noradrenaline determinations were made on 26 and adrenaline determinations on 23 24-hour urine samples collected from this group of women (Table 12). The daily urinary noradrenaline excretion varied between 15.4 and 46.6 μg/24 hrs., with a mean excretion of 24.9 (S. E. 1.75) μg/24 hrs., which is almost the same

Table 10.

Daily excretion of noradrenaline and adrenaline by women from 17 to 29 years old.

Subject No.	Age	Weight kg	Urine volume ml	Noradrenaline µg	Adrenaline µg	Per cent adrenaline
244	17	70	1075	17.7	2.4	11.9
373	18	59	1400	22.4	5.4	19.4
377	18	50	1190	24.0	6.7	21.8
342	19	58	860	15.3	0.6	3.8
370	19	57	720	14.5	1.7	10.5
71	20	57	1050	22.1		
73	20	65	840	14.3		
66	21	62	950	22.1		
67	21	55	1000	35.5		
348	21	53	585	35.8	6.2	14.8
176	21	65	1275	24.0	4.4	15.5
177	21	57	640	16.9	6.9	29.0
178	21	57	505	18.5	2.3	11.1
179	21	58	870	18.4	4.3	18.9
180	21	60	670	25.2		
68	22	65	1040	25.0		
69	22	68	1200	19.0		
70	22	57	1640	27.3		
257	22	54	750	29.1	3.6	11.0
349	22	54	675	36.7	7.6	17.2
383	23	62	1630	30.6	5.4	15.0
396	23	52	1138	25.7	3.7	12.6 [1]
376	24	54	665	26.6	5.3	16.6
385	25	55	520	18.1	3.4	15.8
350	25	65	1330	42.4	7.9	15.7
375	25	55	640	19.5	6.0	23.5
397	26	60	1271	26.1	5.8	18.2 [2]
65	26	60	1100	22.0		
74	26	57	860	17.2		
347	26	70	520	21.0	3.2	13.2
351	26	74	1640	22.4	3.8	14.5
246	27	71	1200	16.1	2.9	15.3
367	27	64	820	12.5	2.1	14.4
72	27	58	1150	23.0		
366	28	64	990	13.5	2.7	16.7
352	28	66	1060	28.5	3.8	11.8
371	28	60	610	17.9	2.0	10.0
Mean	23	60.2	975	22.88	4.23	15.3
S. D.			319	6.90	1.94	4.9
S. E.			52	1.14	0.38	1.0

[1] 6 days. [2] 6 days.

Table 11.

Daily excretion of noradrenaline and adrenaline by men from 30 to 59 years old.

Subject No.	Age	Weight kg	Urine volume ml	Noradrenaline µg	Adrenaline µg	Per cent adrenaline
398	32	70	1200	16.3		
186	35	63	860	18.3	2.1	10.3
32	36	70	900	27.8		
190	36	62	1100	14.1	5.1	26.5
90	37	70	1350	25.4		
206	38	79	970	13.6	6.5	32.3
188	39	70	1150	13.8	3.2	18.8
86	39	78	1490	16.6		
25	40	70	1220	19.4		
189	40	80	750	12.8	3.5	21.2
210	40	70	1200	20.4	8.8	30.1
212	40	65	1050	49.4	12.6	20.3
208	42	90	750	24.8	11.7	32.1
185	42	73	1300	37.5	4.4	10.5
87	42	78	700	19.8		
34	43	79	1450	13.8		
207	44	88	920	27.8	10.5	27.4
187	45	75	1240	32.0	2.7	7.8
82	46	71	2500	28.8		
83	46	83	1100	26.8		
89	46	71	1500	27.5		
205	46	76	1900	35.2	11.0	23.9
3	47	74	748	22.7	3.3	12.7 [1]
182	48	86	860	36.8	3.5	8.7
191	48	63	1140	27.6	6.2	18.6
203	48	65	1100	12.0	3.8	24.1
213	50	85	1075	40.9	8.9	17.6
27	51	68	1700	14.5		
84	52	103	805	28.1		
211	52	69	675	21.6	7.8	26.5
49	56	85	1190	42.0		
183	56	68	760	45.6	8.8	16.2
204	57	80	900	25.9	4.7	15.3
85	59	66	1240	27.8		
88	59	57	1100	20.7		
184	59	78	750	20.5	7.0	25.5
Mean	45	74.4	1129	25.24	6.48	20.3
S. D.			372	9.77	3.22	7.6
S. E.			62	1.63	0.70	1.7

[1] 3 days.

Table 12.

Daily excretion of noradrenaline and adrenaline by women from 30 to 59 years old.

Subject No.	Age	Weight kg	Urine volume ml	Noradrenaline μg	Adrenaline μg	Per cent adrenaline
372	30	73	1350	21.6	2.4	10.0
243	30	75	1260	16.6	3.1	15.7
130	31	63	1200	15.4	5.3	25.6
318	31	66	1250	23.5	3.8	13.9
259	32	66	740	25.5	2.9	10.2
384	32	68	1120	18.0	2.3	11.3
256	33	62	500	36.9	2.3	5.9
132	33	51	650	23.8	6.1	20.4
245	37	72	1550	16.1	2.9	15.3
345	40	94	1100	17.1	2.4	12.3
369	42	83	1240	19.0	5.3	21.8
262	42	52	1650	25.2	10.3	29.0
261	43	69	1200	22.5	5.4	19.4
124	48	78	990	30.0		
247	50	82	1100	22.6	2.9	11.4
399	50	80	1039	22.8	2.7	10.6 [1]
346	52	65	1520	36.6	2.7	6.9
127	54	91	525	34.9		
260	55	70	885	22.8	4.0	14.9
258	58	65	1220	46.6	9.5	16.9
128	58	71	1450	25.3		
Mean	42	71.2	1121	24.89	4.24	15.1
S. D.			320	8.03	2.39	6.2
S. E.			70	1.75	0.56	1.5

[1] 6 days.

as the value for the men of the same age group. The mean adrenaline excretion for the women was 4.2 (S.E. 0.56) μg/hrs., and the range 2.3 to 10.3 μg/24 hrs. The adrenaline percentage was 15.1.

This age group comprises a fairly long period, 30 years. A closer examination made by dividing the group into subgroups revealed that these subgroups did not differ appreciably from the group as a whole in respect of the 24-hour excretion of noradrenaline (Fig. 4).

e. Age group over 60 years. The total number of persons in this group was 57.

The men and women were all inmates of institutions for the aged. Only healthy persons were chosen for the study. The selection was made on the basis of past histories and notes of the physician of the institution. No one who was under medical supervision or who regularly took medicine was included in this group. All the persons from whom the daily urine samples were obtained were regularly afoot during the daytime and performed light duties.

The average age of the men was 76 years, the oldest being 96 years old. The average weight of these men was 70.1 kg, and hence they weighed less on the average than the men in the other age groups.

Noradrenaline determinations were made on 31 and adrenaline determinations on 13 24-hour urine samples from the males of this age group (Table 13). The amount of noradrenaline excreted daily varied from 7.0 to 38.5 μg with a mean of 20.3 (S. E. 1.41) μg/24 hrs., which is the smallest mean value found for groups of adult persons in this study. Also the mean adrenaline excretion was slightly smaller for this group of male persons than for the other groups of men. The mean excretion was 4.8 (S. E. 0.59) μg/24 hrs. The adrenaline percentage was 20.0.

The average age of the women of this group was 72 years, the oldest being 96 years. The average weight of the women of this group was 76.0 kg, which is the highest of the average weights of the present groups. The women in this age group were on average 6 kg heavier than the men.

The determinations of noradrenaline were made on 26 24-hour urine samples and those of adrenaline on 20 24-hour urine samples collected from the women (Table 14). The amount of noradrenaline excreted was greater than for the men, the mean daily excretion being 24.9 (S.E. 2.13) μg/24 hrs. and the range 10.5 to 56.0 μg/24 hrs. The mean excretion of adrenaline was 4.2 (S.E. 0.36) μg/24 hrs. and the range 1.9 to 6.8 μg/24 hrs. The mean adrenaline percentage was 15.4 per cent.

Table 13.

Daily excretion of noradrenaline and adrenaline by men from 60 to 96 years old.

Sample No.	Age	Weight kg	Urine volume ml	Noradrenaline µg	Adrenaline µg	Per cent adrenaline
52	60	70	1300	13.9		
38	63	75	1240	24.8		
42	67	75	740	17.3		
26	67	70	600	39.3		
157	67	60	760	7.0	1.9	21.3
166	67	54	1275	22.4	5.2	18.8
158	68	70	525	19.4	5.4	21.8
35	68	60	1280	21.6		
156	70	51	310	12.4	4.5	26.6
154	71	70	1060	20.2	2.1	9.4
46	73	75	1140	26.1		
163	74	96	1940	37.5	7.8	17.2
44	74	78	600	14.1		
37	76	60	930	14.7		
45	76	90	1250	14.8		
50	77	80	1450	16.9		
48	78	70	730	16.8		
165	79	65	1550	16.7	4.9	22.7
36	81	70	560	20.3		
39	82	62	1840	13.8		
47	82	70	700	23.0		
53	82	90	920	21.7		
159	82	70	1225	23.3	4.1	15.0
164	83	64	1450	10.2	3.9	27.7
162	84	77	1250	25.0	6.0	19.4
161	84	62	275	38.5	9.4	19.6
40	84	70	2100	18.4		
41	85	85	1310	30.9		
155	85	63	875	11.7	4.4	27.3
153	89	55	570	18.8	2.7	12.6
51	96	65	1260	19.1		
Mean	76	70.1	1065	20.34	4.79	20.0
S. D.			457	7.84	2.11	5.6
S. E.			82	1.41	0.59	1.5

Table 14.

Daily excretion of noradrenaline and adrenaline by women from 60 to 96 years old.

Sample No.	Age	Weight kg	Urine volume ml	Noradrenaline μg	Adrenaline μg	Per cent adrenaline
139	62	89	575	20.7	2.3	10.0
143	62	85	940	16.7	3.1	15.7
126	63	94	1150	27.9		
123	66	59	990	20.3		
148	67	76	1300	25.2	6.2	19.7
125	68	67	750	25.3		
131	68	80	1760	18.4		
147	68	78	1650	30.6	6.8	18.2
149	69	86	760	11.9	1.9	13.8
119	69	112	1390	56.0		
151	70	75	1550	27.3	5.1	15.7
152	70	60	1400	28.0	5.6	16.7
121	71	72	1600	19.2		
144	71	85	1275	12.8	3.6	22.0
141	72	69	1020	33.8	3.3	8.9
136	73	75	1250	34.8	6.2	15.1
145	75	77	1315	26.3	3.2	10.8
137	75	81	1175	15.5	4.4	22.1
140	75	79	850	15.5	3.0	16.2
135	76	87	1160	39.5	5.4	12.0
134	76	85	1820	43.4	4.6	9.6
133	76	65	1500	36.0	4.4	10.9
138	77	51	700	20.0	6.8	25.4
146	81	68	860	16.9	2.0	10.6
142	85	70	220	10.5	2.0	16.0
150	96	50	1150	14.1	3.2	18.5
Mean	72	76.0	1158	24.87	4.16	15.4
S. D.			385	10.90	1.63	4.6
S. E.			75	2.13	0.36	1.0

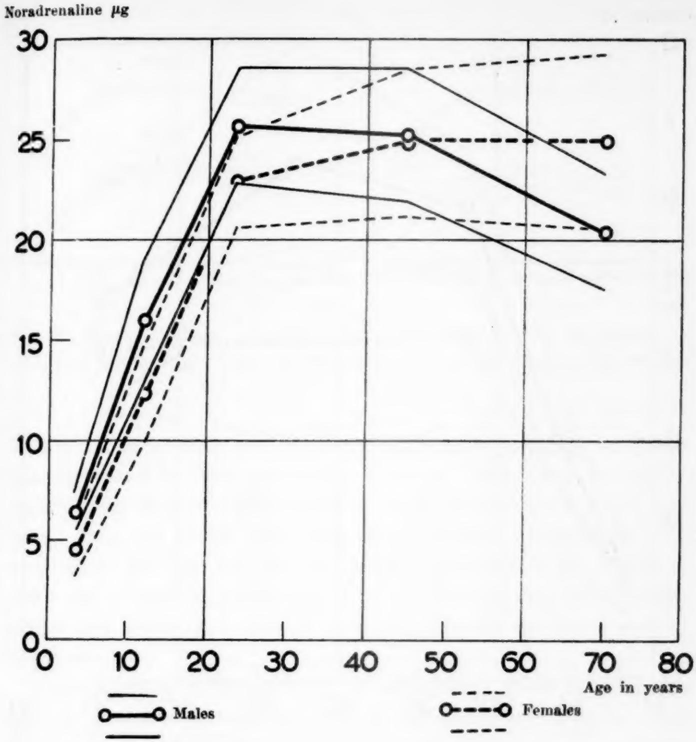

Fig. 4. Mean 24-hour excretion of noradrenaline in different age groups. The mean excretions for the different age groups and their 95 per cent confidence limits have been joined by straight lines.

The mean excretion in different age groups.

To illustrate the variation of the amounts of noradrenaline and adrenaline excreted daily with the ages of the subjects, the mean excretions during 24 hours are given in Figs. 4 and 5 separately for the men and women in each group; the 95 per cent confidence limits are also indicated in the figures.

It will be seen from Fig. 4 that the daily noradrenaline excretion increases from 6.4 μg for the boys from 1.5 to 6 years to

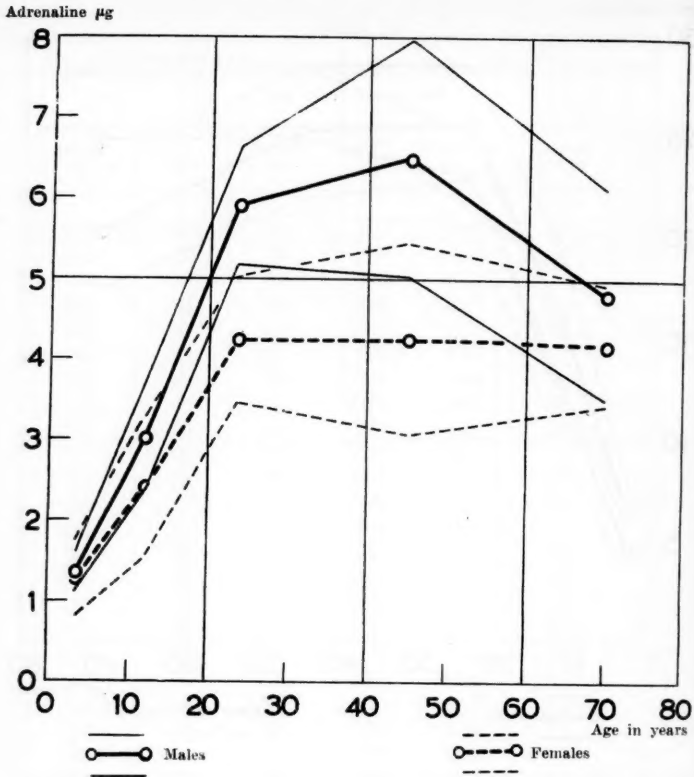

Fig. 5. Mean 24-hour excretion of adrenaline in different age groups. The mean excretions for the different age groups and their 95 per cent confidence limits have been joined by straight lines.

about double this amount for the boys from 7 to 16 years. The excretion which is 4.5 μg for the young girls increases to twice this amount for the girls from 7 to 16 years. The noradrenaline excretion continues to increase for both sexes up to the age group from 17 to 29 years. The mean excretion for the men of the latter group was 25.7 μg, the highest mean excretion found in this study. The amount of noradrenaline excreted is practically the same (25.2 μg) for the group of middle-aged men (30—59 years).

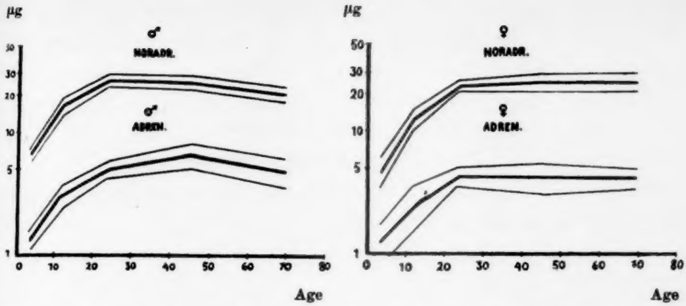

Fig. 6. Mean 24-hour excretion of noradrenaline and adrenaline in different age groups. Data of Figs. 4 and 5 on a semilogarithmic scale.

In the old men (over 60 years) the daily noradrenaline excretion has decreased to 20.3 μg on the average. The variation of the excretion in women differs somewhat from that in men. In the age group from 17 to 29 years, the mean amount of noradrenaline excreted is 22.9 μg, but for the middle-aged women the excretion (24.9 μg) is approximately equal to that for the men of same age group and appears to remain constant also for the older women (24.9 μg).

The variation of the amount of adrenaline excreted with age follows a similar pattern as that of noradrenaline (Fig. 5). The mean daily excretion for the boys and girls of the youngest age group (1.5—6 years) is 1.3 μg. The amount excreted increases with the age and weight slightly more rapidly for the boys than for the girls, the mean excretion for the boys from 7 to 16 years being 3.0 μg and for the girls of same age 2.4 μg/24 hrs. In the age group from 17 to 29 years the mean excretion of adrenaline for men is 5.9 μg/24 hrs. and for the women 4.2 μg/24 hrs. In the middle-aged group the excretion for the men has increased to 6.5 μg, whereas the figure for the women has remained the same (4.3 μg) as in the preceding group of women. The daily adrenaline excretion among the old men is smaller (4.8 μg), but that among the women of the oldest group is the same on the average as in the other adult female groups.

In Fig. 6 the mean daily excretions of noradrenaline and adrenaline are plotted against age on a semilogarithmic scale for both

the female and male groups. The plots show that the ratio of adrenaline to noradrenaline is similar for both sexes and the amounts of adrenaline excreted in different age groups follow fairly closely those of noradrenaline. For all males, the mean ratio of adrenaline to noradrenaline and adrenaline was 19.3 (S.E. 0.6) per cent, and for the females, 16.3 (S.E. 0.6) per cent.

When the 24-hour excretion values for the sexes are combined, the following means are obtained:

Age group	1.5—6	7—16	17—29	30—59	60—96
Noradrenaline					
μg/24 hrs.	5.6	14.5	24.5	25.2	23.1
No. of subjects	*42*	*50*	*85*	*57*	*57*
Adrenaline					
μg/24 hrs.	1.3	2.8	5.1	5.4	4.4
No. of subjects	*26*	*27*	*57*	*39*	*33*

The variation of the excretion per kilogram of body weight.

For the comparison of the variation of the excretion of noradrenaline and adrenaline with age and sex, the excretions of these substances have been computed per kilogram of body weight. This is necessary because the body weights vary greatly with age and sex and hence the absolute amounts excreted as found by analysis are not directly comparable. The mean daily excretion of noradrenaline and adrenaline per kilogram of body weight is shown for the different age groups in Table 15. To assess the differences between the age groups and sexes, the data have been subjected to analysis of variance.

a. The statistical treatment revealed that the differences in the amounts of noradrenaline excreted daily per kilogram of body weight in different age groups are significant. The excretion of noradrenaline per kilogram of body weight is highest in the age group from 7 to 16 years for both sexes. For the boys it is 0.486 μg/kg/24 hrs. and for the girls 0.417 μg/kg/24 hrs. The excretion decreases with increasing age. The decrease is greater for the men than for the women when passing to the next age group (17 to 29

Table 15.

Twenty-four-hour urinary excretion of noradrenaline and adrenaline calculated per kilogram of body weight.

Age group	Noradrenaline μg			Adrenaline μg		
	Mean	S. D.	S. E.	Mean	S. D.	S. E.
Males						
1.5 — 6	0.399	0.124	0.023	0.0804	0.0256	0.0062
7 — 16	0.476	0.175	0.032	0.0867	0.0407	0.0093
17 — 29	0.359	0.144	0.021	0.0815	0.0288	0.0052
30 — 59	0.353	0.127	0.022	0.0870	0.0425	0.0093
60 — 96	0.291	0.107	0.019	0.0728	0.0308	0.0085
	0.373			0.0823		
Females						
1.5 — 6	0.309	0.127	0.037	0.0829	0.0394	0.0131
7 — 16	0.407	0.153	0.034	0.0719	0.0256	0.0091
17 — 29	0.387	0.132	0.022	0.0720	0.0357	0.0070
30 — 59	0.360	0.138	0.030	0.0654	0.0472	0.0111
60 — 96	0.327	0.123	0.024	0.0580	0.0279	0.0062
	0.364			0.0683		

years). The smallest excretion per kilogram was found for the men over 60 years old. It may also be noted that although the absolute amount of noradrenaline excreted was higher for the oldest women than for the younger women, the amount excreted per kilogram is significantly, though not much, smaller than for the other adult women.

b. The variation of the amount of adrenaline excreted daily per kilogram of body weight was not found to be significantly different among the various male age groups. Neither was a statistically significant difference established between the different female age groups, although the daily excretion per kilogram of body weight underwent an apparent decrease with age.

c. One of the questions for which an answer was sought was whether there is any difference between the sexes in the secretion of noradrenaline and adrenaline. No significant differences were found to exist between the amounts of noradrenaline excreted per

kilogram of body weight by the two sexes in the different age groups (the probabilities P exceeded 0.05).

d. Although the absolute mean daily excretion of adrenaline seemed greater for the males than for the females in all age groups, and although the mean excretion calculated per kilogram of body weight was 0.082 μg for the men and 0.068 μg for the women (Table 15), the statistical analysis showed that it is not possible to say that the excretion of adrenaline per kilogram is significantly greater for the men than for the women.

The noradrenaline content of the urine.

The volumes of urine excreted by children are relatively large compared with the volumes excreted by adults (NELSON 1950). This was also clearly seen in the present study, since the mean volume of urine was 1220 ml for the boys from 7 to 16 years and 1050 ml for the girls of the same age group. These volumes are equal to or even greater than the volumes excreted by adults; the figure for the boys was the highest found in the present study.

When the daily excretion of urine is computed per kilogram of body weight, the difference between children and adults becomes even more clearly evident. The mean daily excretion per kilogram is shown for the different age groups in Fig. 7. The mean figures for both the girls and boys in the two age groups from 1.5 to 6 and from 7 to 16 years are about twice those for the adults. The mean excretion for children from 1.5 to 6 years is 34 ml/kg, approximately the same for girls from 7 to 16 years, and slightly higher, 38 ml/kg, for the boys of the latter age group. The excretion remains fairly constant at about 16 ml/kg for the adults. The curves plotting the excretion of urine with age for the two sexes run parallel.

Since the amounts of noradrenaline and adrenaline excreted daily by children are small, only one-fourth (for the children 1.5 to 6 years old) and about one-half (for the children 7 to 16 years old) of the amounts excreted by adults, and the volumes of urine large, the concentrations of noradrenaline and adrenaline are considerably lower in the urine of children than of adults. The concentration of noradrenaline in urine of children from 1.5 to 6

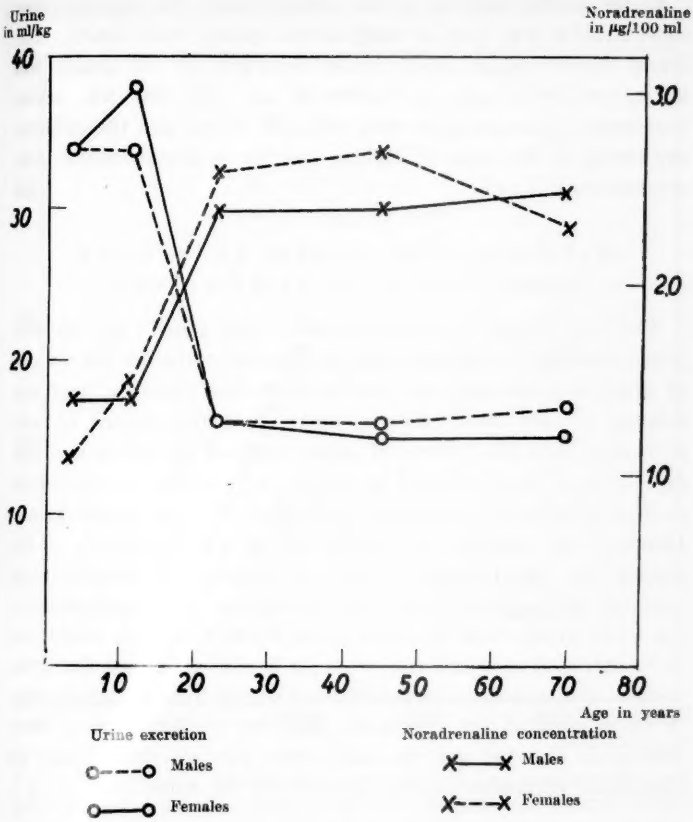

Fig. 7. Mean 24-hour excretion of urine per kilogram of body weight and the mean noradrenaline concentration in different age groups.

years old varied from 0.3 to 3.1 µg per 100 ml, with a mean of 1.1 µg/100 ml for the girls and 1.4 µg/100 ml for the boys (Fig. 7). In the group from 7 to 16 years old, the noradrenaline concentration varied from 0.6 µg to 4.0 µg per 100 ml, the mean for the girls being 1.5 µg per 100 ml and for the boys 1.4 µg per 100 ml. The noradrenaline concentration in the urine was fairly level for the adults of the different age groups; the mean values varied from 2.3 to 2.7 µg per 100 ml (Fig. 7).

In the healthy subjects of the present study, the noradrenaline concentration was seen to vary within rather wide limits. The lowest noradrenaline concentration recorded for the adults was 0.7 μg per 100 ml and the highest 14 μg per 100 ml, which represents a concentration ratio of 1 : 20. When also the children are included, the ratio of highest and lowest noradrenaline concentrations is 1 : 47.

Relation of the amount of excreted noradrenaline to urine volume.

Since the volume of urine excreted varied greatly for the subjects examined, a study was made to determine whether the volume of urine excreted daily and the noradrenaline excretion bear any relation to each other. As seen above, both the amount of noradrenaline and the volume of urine excreted by children differ greatly from those excreted by adults and also the noradrenaline content of urine is much lower for children than for adults. It was therefore not possible to include the group of children in the comparison. The relation between the amounts of noradrenaline excreted daily and the daily urine volume was hence examined for the adult group from 17 to 29 years. From Fig. 8, in which the daily excretion of noradrenaline in μg is plotted against the urine volume, it is seen that the points are scattered over the whole area. A calculation of the regression lines for the amounts of noradrenaline excreted and the daily urine volumes showed that no significant regression existed for either of the sexes.

B. THE DIURNAL VARIATION OF THE EXCRETION OF NORADRENALINE AND ADRENALINE.

The physical and psychic activity of a human being leading a normal life varies greatly at different times of the day. It may be assumed that, compared with periods of rest, the increased activity during daytime leads also to an augmented function of the sympathetic nervous system and the suprarenal medulla. The variation of the activity can be followed by examining the urinary excretion of noradrenaline and adrenaline at different periods of the day.

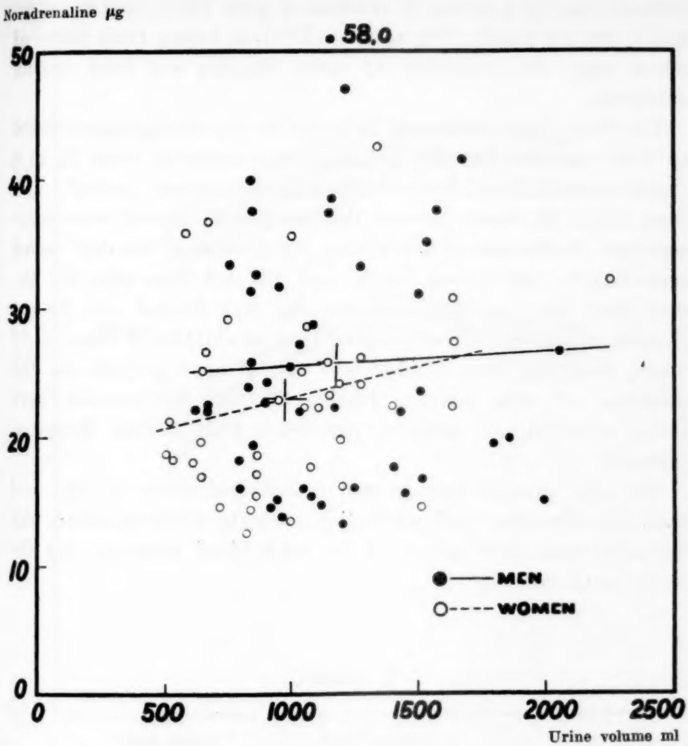

Fig. 8. The relation between the 24-hour excretion of noradrenaline and urine volume in men and women from 17 to 29 years old. The regressions are not significant.

1. Material.

A group of 12 persons were included in this study. Five were young healthy conscripts between 20 and 21 years of age, whose physical activities and other daily habits were consequently similar. The amounts of noradrenaline and adrenaline excreted by these men at different periods of the day were evaluated, in the case of four of these men during 96 hours, and in one case (N. T.) during 48 hours. The excretion of these compounds was also followed in a group of 3 female laboratory

workers, and in a group of children, 3 girls (10, 9 and 8 years) and 1 boy (3 years). The children lived at home; from those of school age, the collection of urine samples was done during vacations.

The urine excreted during 24 hours by the adults was collected in three samples. For the men, the urine excreted from 22 to 6 (night period), from 6 to 14 (first daytime 8-hour period), and from 14 to 22 o'clock (second daytime 8-hour period) were kept separate. In the case of the women, the division of the day varied according to their living habits, and differed from that for the men, but also in these cases the day was divided into 8-hour periods. Since the normal sleeping time of children is close to 12 hours, their day was divided into two 12-hour periods for the collection of urine samples. During the time the samples were being collected, all subjects performed their routine duties as normally.

The urine samples were treated as described above, (p. 20), and their noradrenaline and adrenaline contents were estimated biologically from their action on the cat's blood pressure and the hen's rectal caecum.

2. Results.

Men.

The number of urine samples from the men which were examined was 53. Noradrenaline and adrenaline were both found to be present in all samples, although their contents in the night samples were definitely lower than in the day samples. The mean amounts of noradrenaline and adrenaline excreted during different periods by the different subjects are given together with the ranges in Table 16.

Eight-hour night period. The amounts of noradrenaline excreted during the 8-hour night period are seen to vary from 1.4 to 8.4 μg, but the mean excretions for the different persons vary from 4.4 to 6.6 μg. The mean for all the men was 5.7 μg (Fig. 9). The smallest amount of adrenaline found for the men was 0.4 μg and the highest 2.2 μg. The mean excretion for the five men was

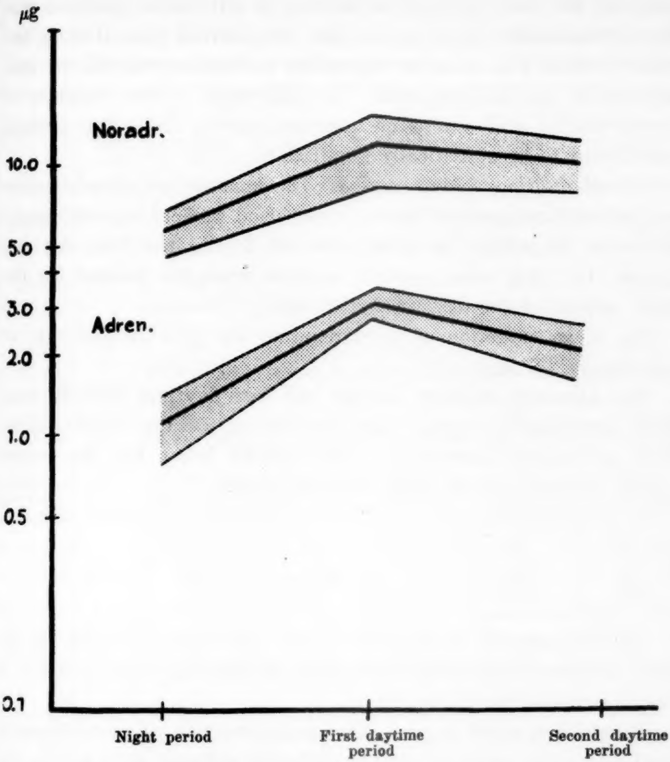

Fig. 9. The excretion of noradrenaline and adrenaline by men during different periods of the day. The excretions during periods from 3 to 5 days of five subjects have been averaged. The means for the different periods and their 95 per cent confidence limits have been joined by straight lines. Semilogarithmic scale.

1.1 μg. The mean amount of adrenaline excreted was 16 per cent of the total amount of noradrenaline and adrenaline.

First daytime 8-hour period. The amount of noradrenaline excreted was in all cases higher during this period than during the 8-hour night period. The same was true for adrenaline. The average amounts excreted by the men were 11.9 μg of noradrenaline and 3.1 μg of adrenaline; for noradrenaline the increase was

thus 100 per cent, whereas the amount of adrenaline excreted was three times higher during the first day period than during the night period. The ratio of adrenaline to total noradrenaline and adrenaline was 20.8 per cent. The difference in the amounts of noradrenaline and adrenaline excreted during these two periods was found to be statistically significant.

Second daytime 8-hour period. The amounts of noradrenaline and adrenaline excreted during this period were of approximately the same magnitude as those excreted during the first daytime period. In a few cases, a slight decrease from the amount for the early period of the day was established.

The mean excretion of noradrenaline was 10.3 μg and that of adrenaline 2.2 μg.

The amounts excreted during the two daytime periods were both significantly higher than the values for the night period. The adrenaline percentage was slightly lower for the second (17.9 %) than for the first daytime period.

Women.

The amounts of noradrenaline and adrenaline excreted by the three adult women were followed for 6 days. The total number of urine samples tested was 54.

Eight-hour night period. The mean amounts of noradrenaline and adrenaline excreted by the different subjects were nearly the same (Table 16). The mean excretion of noradrenaline for the group, 5.0 μg, was almost equal to that for the men (Fig. 10). The amounts of adrenaline excreted varied greatly, even for the same subject, on different nights. The amount was sometimes as low as 0.1 μg. The mean adrenaline excretion for the women during the night period was 0.7 μg. The adrenaline percentage was 12.3.

First 8-hour daytime period. Similarly as for the men, the excretion of noradrenaline and adrenaline was higher for the women during this period than during the night period. In a few cases, the amount of noradrenaline excreted was up to 3 times greater during this period than during the night period, but the mean increase was also about 100 per cent for the women. Since the amount of adrenaline excreted during the night was often

Table 16.

Diurnal urinary excretion of noradrenaline and adrenaline in men and women. The mean excretions and ranges are given for three 8-hour periods.

Subject	No. of days	Night period		First daytime period		Second daytime period	
		Noradren. µg	Adren. µg	Noradren. µg	Adren. µg	Noradren. µg	Adren. µg
E. T., male, age 20, 68 kg	4	4.4 (1.4—8.4)	0.9 (0.4—1.7)	9.6 (5.5—18.2)	3.2 (1.4—7.6)	8.5 (6.9—10.1)	2.4 (1.0—3.4)
S. M., male, age 20. 75 kg	4	6.0 (3.9—8.7)	1.0 (0.7—1.2)	10.9 (6.4—16.3)	2.7 (1.8—3.1)	8.9 (7.0—11.5)	1.7 (0.9—2.6)
O. P., male, age 21, 71 kg	4	6.6 (5.3—7.6)	0.9 (0.4—1.7)	13.7 (8.3—20.0)	3.4 (2.4—4.5)	12.9 (8.0—16.5)	2.5 (1.4—3.3)
U. V., male age 21, 70 kg	4	6.0 (4.8—7.0)	1.5 (1.2—2.2)	9.2 (7.0—10.8)	2.7 (1.8—4.0)	10.9 (6.0—14.7)	2.5 (1.6—4.2)
N. T., male, age 20, 71 kg	2	5.6 (3.1—8.1)	1.0 (0.5—1.5)	16.3 (13.1-19.5)	3.4 (2.7—4.1)	9.3 (6.8—11.7)	1.6 (1.6—1.7)
Mean		5.7	1.1	11.9	3.1	10.1	2.1
S. D.		0.95	0.27	2.93	0.29	1.85	0.38
S. E.		0.42	0.12	1.36	0.13	0.83	0.17
A.S., female, age 23, 52 kg	6	5.6 (4.2—7.5)	0.6 (0.2—1.0)	10.3 (7.8—14.5)	1.8 (1.3—2.8)	8.2 (5.4—10.3)	1.3 (1.0—1.8)
E.L., female, age 26, 60 kg	6	4.3 (2.8—5.4)	1.1 (0.6—1.7)	11.4 (7.7—15.6)	2.6 (1.8—3.1)	10.4 (7.2—14.3)	2.2 (1.2—2.9)
N.B., female, age 50, 80 kg	6	5.2 (3.5—7.8)	0.5 (0.1—1.8)	10.7 (7.8—12.7)	1.3 (0.5—3.2)	6.9 (5.0—10.1)	0.9 (0.5—1.5)
Mean		5.0	0.7	10.8	1.9	8.5	1.5
S. D.		0.62	0.24	0.52	0.62	1.68	0.62
S. E.		0.44	0.17	0.38	0.44	1.19	0.44

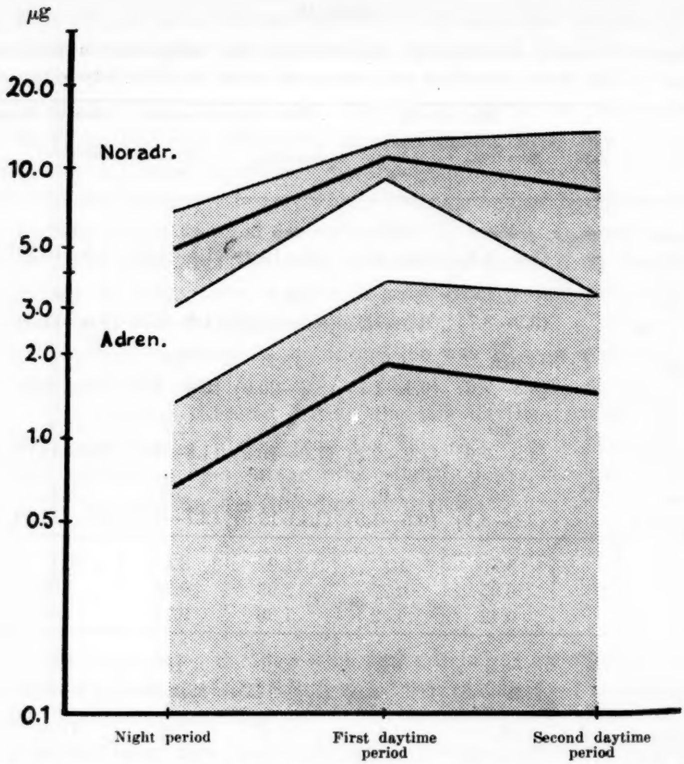

Fig. 10. The excretion of noradrenaline and adrenaline by women during different periods of the day. The excretions during 6 days of three subjects have been averaged. The means for the different periods and their 95 per cent confidence limits have been joined by straight lines. Semilogarithmic scale.

small, the increase was in some cases from 12- to 15-fold but the average excretion was about threefold. The mean excretions for the women were 10.8 μg of noradrenaline and 1.9 μg of adrenaline; the excretion of both amines was significantly larger during this period than during the night period. The mean adrenaline percentage was 15.0.

Table 17.

Diurnal urinary excretion of noradrenaline and adrenaline in children. The mean excretions and ranges are given for two 12-hour periods.

Subject	No. of days	Night period		Day period	
		Noradren. μg	Adren. μg	Noradren. μg	Adren. μg
T. boy age 3, 17 kg	12(9) [1]	1.8 (0.9—2.4)	0.4 (0.1—0.8)	4.4 (3.3—6.9)	1.2 (0.7—2.0)
M. girl age 8, 25 kg	8 (6)	3.3 (2.2—5.6)	0.5 (0.3—0.7)	6.9 (5.3—10.8)	1.4 (1.1—1.7)
A. girl age 9, 30 kg	9 (8)	4.6 (2.8—6.2)	0.5 (0.2—0.9)	10.5 (8.0—14.9)	1.6 (1.0—2.3)
P. girl age 10, 33 kg	2	5.8 (5.5—6.2)	0.6 (0.5—0.6)	11.6 (11.5-11.7)	1.5 (1.1—1.9)
Mean		3.9	0.5	8.3	1.4
S. D.		1.84	0.1	3.36	0.20
S. E.		0.92	0.05	1.68	0.10

[1] Figures in brackets relate to adrenaline.

Second 8-hour daytime period. The mean noradrenaline excretion for the women was 8.5 μg and that of adrenaline 1.5 μg. These values are almost as high as those for the first day period, although as in the case of the men, the amounts tended to be slightly smaller during the second than during the first daytime period. The adrenaline percentage was 14.4.

Statistical evaluation of the results revealed that the amounts of noradrenaline and adrenaline excreted during the two daytime periods were significantly greater than those excreted during the night, while the difference between the two daytime periods was not significant.

Children.

The daily variation of noradrenaline and adrenaline excretion was followed in the 4 children by determining the noradrenaline content in 62 urine samples and the adrenaline content in 49 urine

samples which had been collected during two 12-hour periods of the day.

Twelve-hour night period. The data in Table 17 and Fig. 11 show that the amount of noradrenaline excreted during the night increases with the age of the child. The mean noradrenaline excretion for the 3-year-old boy was 1.8 μg. For the 8-year-old girl the excretion was nearly twice this amount. The mean excretions for the girls of 9 and 10 years, 4.6 and 5.8 μg, were almost as high as those found above for the adult subjects. It should be noted, however, that the periods during which the urine was collected were 4 hours longer for the children than for the adults. The mean adrenaline excretions of the children during the night period were 0.4, 0.5, 0.5, and 0.6 μg. These amounts are smaller than those for the adults. The increase of the adrenaline excretion with age is not so clear as in the case of noradrenaline. The mean adrenaline percentage for the children during the night period was 12.1.

Twelve-hour daytime period. The amounts of noradrenaline and adrenaline excreted during the day period were on all days greater than the amounts excreted during the night period (Table 17, Fig. 11). For the three-year-old boy the mean noradrenaline excretion, 4.4 μg, was more than twice the amount excreted during the night period. Similar increases were found also for the other children. The excretions during the day period were 6.9, 10.5 and 11.6 μg for the girls of 8, 9 and 10 years, respectively.

The adrenaline excretion underwent a relatively greater increase than the noradrenaline excretion. The mean excretions of adrenaline during the day period were 1.3 μg, 1.4 μg, 1.6 μg and 1.5 μg. The adrenaline percentage was higher during the day, 16.1 per cent, than during the night, 12.1 per cent.

C. DISCUSSION.

When the amounts of noradrenaline and adrenaline excreted in the urine in infusion experiments are compared with the amounts administered, it is possible to infer to some extent the amounts liberated in the blood from the amounts in the urine. The amounts released from the nerve endings in the organism

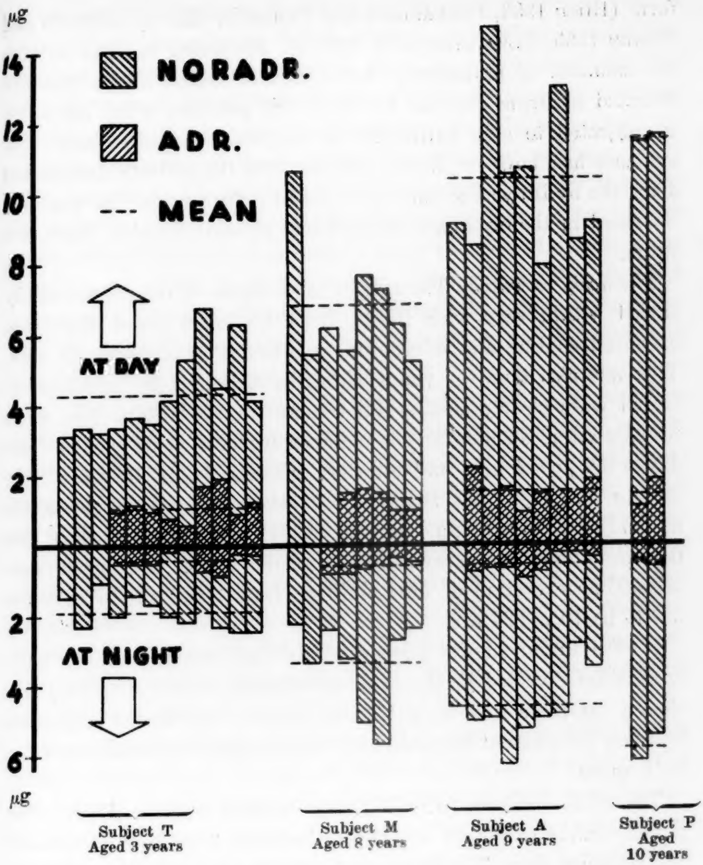

Fig. 11. The excretion of noradrenaline and adrenaline by four children during 12-hour day and night periods. See Table 17.

cannot, however, be deduced from the urinary excretion since a large part of these hormones may decompose before they enter the blood stream. However, it seems probable that the amounts found in urine reflect also the amounts liberated in the organism.

A part of the noradrenaline and adrenaline in the urine has been shown to be present in conjugated and biologically inactive

form (BURN 1953, PEKKARINEN and PITKÄNEN 1955 b, v. EULER and ORWÉN 1955, ELMADJIAN et al. 1956 a). According to these authors the amounts of biologically active noradrenaline and adrenaline detected in urine increase by 50 to 150 per cent when the urine is subjected to acid hydrolysis or enzymatic decomposition. No evidence has, however, been presented that the activity determined after the hydrolysis of the urine would reflect better the amounts liberated in the organism and is hence of more reliable diagnostic value.

The daily excretion. The ages of the subjects of the present study varied within very wide limits, from 1.5 to 96 years. Excretion of noradrenaline and adrenaline was found to occur at all ages. This was also true for young children, although the amounts excreted were clearly smaller than those for the adults. The mean noradrenaline output for the children under 7 years varied from 4.5 to 6.5 μg/24 hrs., and for those between 7 and 16 years from 12.3 to 16.0 μg/24 hrs. BURN (1953), who employed the extraction method of v. EULER and HELLNER (1951) and also performed the determinations on unhydrolysed urine, found the mean noradrenaline excretion of nine children from 3 months to 10 years old to be 10 μg/24 hrs.

It was found in the present study that the amounts of noradrenaline excreted in the urine of children from 1.5 to 16 years clearly increases with weight, since a highly significant regression between the amount excreted and body weight was established for both sexes.

The adult subjects (over 17 years of age) numbered 199. The noradrenaline excretion was determined for these over periods of one or more days. The mean excretions for the age groups varied from 20 to 26 μg per 24 hours. These figures are in good agreement with the results obtained by other workers who have used similar methods of estimation. v. EULER, HELLNER-BJÖRKMAN and ORWÉN (1955) found the mean figure for 6 men to be 26 μg/24 hours and for 9 women 27 μg/24 hours. GOLDENBERG et al. (1954) found the excretion of catechol amines in 13 normal subjects to vary from 14 to 41 μg of noradrenaline equivalents per 25 hours. The values obtained using chemical methods of estimation have slightly deviated from those found by biological methods. PEKKARINEN and PITKÄNEN (1955 b) and PITKÄNEN (1956) found, using a fluori-

metric method, the noradrenaline excretion to be 81 μg per 24 hours. Using a modified fluorimetric method, PITKÄNEN (1956) obtained a mean normal excretion of 33 μg per 24 hours for 154 subjects. Also v. EULER and FLODING (1955) developed a chemical method which yielded similar values for the catechol amines excreted in the urine as those obtained by biological methods.

The excretion of adrenaline is also low, about 1 μg per 24 hours, for children under 7 years. The amount excreted is higher for adults, being from 4 to 6 μg per 24 hours for men of different age groups and about 4 μg for women. Using a similar method, v. EULER, HELLNER-BJÖRKMAN and ORWÉN (1955) found the mean free adrenaline excretion for 6 men to be 5 μg per 24 hours and that for 9 women 4 μg per 24 hours.

In this connection it is of interest to note how the excretion of 17-ketosteroids, which primarily originate in the adrenal cortex, varies with age and sex. The excretion is small in children, increases rapidly during puberty and attains a maximum at the age of 25 to 35 years. After the fortieth year, the excretion begins to decrease and this decrease continues rapidly, especially in men (HAMBURGER 1948, KIRK 1949, KINNUNEN, PEKKARINEN and SIMOLA 1952). In respect of the marked difference in excretion between the sexes and the abrupt decrease with increasing age, the excretion of 17-ketosteroids differs clearly from the excretion of noradrenaline and adrenaline.

Since the intention was to compare the excretions in different age groups and sexes in the present study, the groups of subjects did not include persons engaged in heavy physical work, and the subjects were chosen so that their daily activities were as similar as possible. In order to permit a comparison of the excretions in different groups of subjects, the excretions of noradrenaline and adrenaline were also calculated per kilogram of body weight. It was established that the excretion of noradrenaline was greatest among boys and girls in the age group from 7 to 16 years. In the older age groups the excretion per kilogram decreased regularly with age; for example, the excretion of boys under 17 was about 65 per cent greater than that of men over 60. The increase in the mean body weight with age observed in all groups except the group of men over 60 had the result that this difference did not become evident when the absolute 24-hour excretions were compared. In his study

of 38 persons referred to above, BURN (1953) found that older adults excrete more noradrenaline than younger adults. As he did not give the weights of his subjects, it is not possible to determine how the excretion per kilogram varied with age.

No statistically significant differences were found between the age groups in the excretion of adrenaline per kilogram of body weight. This indicates that the adrenal medulla maintains its activity up to a high age. If atrophy occurs in this tissue, which functions as an endocrine gland, the excretion of adrenaline would be expected to decrease. According to v. EULER et al. (1954 b), the removal of the suprarenal glands leads to a marked decrease (80 per cent) in the amount of adrenaline excreted and ELMADJIAN et al. (1956 b) detected no adrenaline in the urine of an adrenectomised subject. HÖKFELT (1951) and SHEPHERD and WEST (1951 a, b) have observed that the adrenal gland of an adult contains relatively more adrenaline than that of a newborn child. In the present study, no children under 1.5 years were examined.

When the noradrenaline excretions per kilogram of body weight for both sexes were compared, no statistical differences were established. Although the execretion of adrenaline per kilogram appeared to be higher for the male groups with the exception of the youngest group than for the female groups, the differences are not statistically significant.

When the results obtained are to be used for comparison when the excretion of a subject is examined, the normal daily noradrenaline output is obtained by multiplying the mean excretion per kilogram for the corresponding age group (Table 15) by the weight of the subject. The variation from person to person is great, but since a daily excretion exceeding 50 μg was observed with only two subjects in the present material, this figure may be considered a limit which the normal excretion seldom exceeds. The normal output of adrenaline for a subject can be calculated in a similar manner by multiplying the mean excretion found (0.075 μg/kg/24 hrs.; Table 15) by the weight of the subject in kilograms. Age or sex need not be taken into account in this case. The individual variation is again large, but a 24-hour excretion of 12 μg was exceeded only in two of the subjects of the present study.

Diurnal Variation. The excretions of noradrenaline and adrena-

line were found to vary with the time of day. The mean excretion at night for a group of 5 men was 5.7 μg, and for a group of 3 women 5.0 μg during 8 hours. In the children, the night excretion increased with age and weight.

The mean excretions for the adults at night were fairly close to each other (Table 16), the noradrenaline excretion being more constant than that of adrenaline. This basal secretion may be considered to indicate that a certain activity prevails in the sympathetic nervous system during periods of rest (sleep) which effects liberation of noradrenaline and its secretion in urine (v. EULER, LUFT and SUNDIN 1955, SUNDIN 1956).

The adrenaline in the urine is believed to originate primarily in the adrenal medulla (v. EULER et al. 1954 b, ELMADJIAN et al. 1956 b). The amounts excreted at night may be very small, even less than 0.1 μg during 8 hours. This and the observation that the relative adrenaline excretion is smaller at night than during daytime implies that the activity of the suprarenal gland is very weak at rest (cf. ELMADJIAN et al. 1956 b and RENTON and WEIL-MALHERBE 1956).

The noradrenaline excretion per unit of time is from 1.5 to 3 times as great during the daytime as at night and is a sign of increased vasomotor activity during the day.

Psychic factors have been found to influence the excretion of adrenaline in the urine. v. EULER and LUNDBERG (1954) observed that pilots have a greater adrenaline excretion when they are flying and ELMADJIAN et al. (1956 b) report that psychic stress may increase the adrenaline content of the urine up to tenfold. Obviously normal daily activities greatly stimulate the adrenaline secretion by the suprarenal medulla, since the excretion during the day was about 3 times the excretion at night (cf. ELMADJIAN et al. 1956 b).

The results of the present study are in agreement with those of v. EULER, HELLNER-BJÖRKMAN and ORWÉN (1955) as far as the diurnal variation is concerned. RAAB and GIGEE (1954), who used chemical methods, were unable to detect any differences in the day and night excretion of catechol amines in hospital patients. When the subject is at rest during both day and night, the variations in the amounts of noradrenaline and adrenaline excreted are slight (v. EULER, LUFT and SUNDIN 1955). The present results

suggest that the excretion of noradrenaline and adrenaline is closely related to physical and mental activity and possibly to the metabolic functions, but the observed rhythm cannot be considered a basic physiological phenomenon to the same extent as, for instance, the variation of the level of 17-hydroxycorticosteroids in the plasma or the variation of the excretion of 17-ketosteroids in the urine during different periods of the day (e.g. Doe et al. 1956, Bliss et al. 1953, Sandberg et al. 1953, Pincus 1943), the rhythm of which remains constant even in night workers and in blind persons (Migeon et al. 1956). The excretion of noradrenaline and adrenaline seems to react more sensitively to the exertions the organism is subjected to during the day.

THE EFFECT OF INCREASED MUSCULAR ACTIVITY ON THE EXCRETION OF NORADRENALINE AND ADRENALINE.

The amounts of noradrenaline and adrenaline excreted by subjects of different ages under normal conditions that were evaluated as described above provide a basis for a comparison of the excretion of these substances by men who perform heavy muscular work. The intention was to determine not only the levels to which the noradrenaline and adrenaline excretions rise due to greatly augmented muscular activity, but also the rates at which the contents of the compounds in the urine rise to and the time they remain at the high levels. For the purpose, the excretion of noradrenaline and adrenaline in urine was studied in subjects who exerted themselves physically in various ways, primarily in various forms of athletics.

The variation of the amount of noradrenaline excreted in the urine by athletes in training was determined by examining urine samples collected before, during, and after practice periods. The effect of exhausting competition of long duration on the excretion of noradrenaline and adrenaline in the urine was studied in the case of 10 marathon runners and 10 long-distance skiers. For the

purpose of determining the effect of very arduous physical work on the noradrenaline and adrenaline excretion, the excretion of these compounds was followed in five participants in a national competition of woodcutters.

A. SPORTS TRAINING.

1. Material.

The subjects were three middle-distance runners who were training for coming sports events. The daily training program during the time the study was made consisted mainly of alternating dashes and gymnastics over a period from 45 to 70 minutes. The program did not deviate in any respect from the usual on those days when urine samples were collected from the runners. Subjects I and II were young athletes and subject III one of the country's best middle-distance runners.

The subjects urinated at a certain specified time from 1.5—2 hours prior to and immediately before the practice period (samples A). The urine excreted during and immediately after the practice was also collected for the study (samples B). A third sample (C) was taken 2 to 2.5 hours after the exercise period had ended. The samples were treated in the manner described on p. 20 and their noradrenaline and adrenaline contents estimated from the action on the cat's blood pressure.

2. Results.

In the case of two of the subjects the excretion of noradrenaline in the urine was followed during four practice periods, and in one case during three practice periods. The total number of samples examined was 31. Since the periods when the urine samples were collected varied somewhat for each subject, the amounts of noradrenaline excreted at various times were calculated per minute. The mean values are given in Table 18 and the changes in the amounts excreted during different periods in Table 19.

The excretion of adrenaline before the practice period varied among the subjects from 0.01 to 0.037 μg/min. (Fig 12). The

Table 18.

The effect of sport practice on the excretion of noradrenaline.

Subject	No. of practice periods	A Before µg/min.	B During µg/min.	C After µg/min.
I	4	0.026	0.108	0.029
II	4	0.014	0.084	0.026
III	3	0.018	0.105	0.043

Table 19.

The differences between the mean noradrenaline excretions given in Table 18.

Subject	Mean increase during practice period (B—A)	Mean decrease after practice period (B—C)
I	0.082 ± 0.0092	0.079 ± 0.0079
II	0.070 ± 0.0010	0.058 ± 0.0106
III	0.087 ± 0.0143	
Mean	0.080 ± 0.0050	0.068 ± 0.0061
t	16.00	11.15
P	< 0.01	< 0.01

excretion at rest was 0.026 μg/min. for subject I, 0.014 μg/min. for subject II, and 0.018 μg/min. for subject III. From these figures, assuming the overnight excretion to be 5 to 6 μg, the daily excretion of noradrenaline is found to vary from 20 to 30 μg for the three subjects. The amounts are in agreement with the values found above for young men (p. 44) and with those reported by v. Euler and Hellner (1951).

During the practice period, the excretion (in samples B) varied from 0.061 to 0.145 μg of noradrenaline per minute. In some practice periods the noradrenaline excretion per minute was ten times the excretion before the practice period (Fig. 11). The aver-

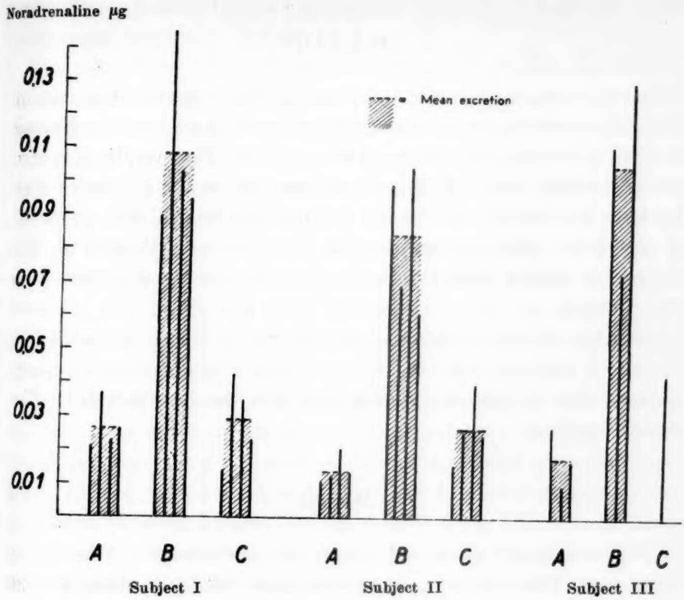

Fig. 12. The excretion of noradrenaline by three athletes before (A), during (B) and after (C) practice periods.

age increase was from four- to sixfold: 4.1 times for subject I, 6.0 times for subject II, and 5.7 times for subject III. The smallest mean noradrenaline excretion (0.084 μg/min.) was recorded for the subject whose resting excretion was the smallest (0.014 μg/min.). The mean increase in the noradrenaline excretion per unit of time resulting from the practice was statistically significant ($P < 0.01$) for all subjects as a group.

The noradrenaline excretion per minute was in all cases lower 2 hours after the practice periods than during these periods. The decreases were statistically significant (Table 19). The mean excretion for all subjects was slightly higher after than before the practice period (Fig. 12).

B. EXHAUSTING COMPETITION OF LONG DURATION.

This investigation was conducted in Turku in the Autumn of 1954 in connection with the Turku marathon and in the Spring of 1955 in connection with a ski competition. The marathon course was of normal length, 42.2 km, and was run on the highways near Turku. The length of the ski competition was 40 km. Several of the best marathon runners of Finland participated in the marathon, among them the present and the past world champion title holders.

Since the marathon and ski competitions were run under similar conditions and corresponded to each other also otherwise in many respects, the method of investigation and the results will be discussed together.

1. Material.

All the subjects examined, 10 marathon runners and 10 skiers, were men. The competitors emptied their bladders about 20—30 min. before the actual race. Immediately after they had come to the finish, they went to a Finnish steam bath. It was found that in most cases the bladder was relaxed immediately after the race, and hence the volume of urine excreted was small and urination difficult. For this reason, the men were given time, about 15 to 20 min., to recover before the urine was collected. Each was requested to empty his bladder as completely as possible. Catheterization was not performed.

The urine extracts were made from 25-ml samples in the usual manner and analysed on the same or the following day.

The competitors from whom the samples were taken were not selected by taking into account their condition after or their final placing in the races.

2. Results.

In the case of both groups of competitors, the volume of urine excreted after a competition of $2\frac{1}{2}$ to 3 hours' duration was very small, and varied from 40 to 180 ml. The urine was dark in color

and often opaque. The pH of the sample was generally low, in most cases between 5 and 6.

The tests showed that the amounts of noradrenaline and adrenaline secreted during the race were high. The amounts excreted and the adrenaline percentage are given for each competitor in Table 20. The noradrenaline excreted by the marathon runners varied from 12 to 31.5 μg, with a mean of 21.7 μg, while that excreted by the skiers varied from 8.1 to 51 μg, with a mean of 21.7 μg. Since the normal excretion of noradrenaline by adults is about 0.017 μg per min., and since the marathon and ski races took from $2\frac{1}{2}$ to 3 hours, the noradrenaline excretion was from 3 to 17 times the normal. The excretion per minute during the race varied from 0.05 to 0.3 μg.

The relative increase in the amount of adrenaline secreted during the races was much larger than that of noradrenaline. Among the marathon runners, the amount of adrenaline excreted varied from 6.1 to 18.9 μg, with a mean of 9.8 μg. The excretion was lower for the skiers, between 3.0 and 10.9 μg, mean 6.6 μg. The normal daily excretion of adrenaline of young men is 5.9 μg, or about 0.004 μg/min. The excretion for the competitors varied from 0.017 to 0.1 μg per minute, and hence the increase was from 4-to 25-fold. The ratio of the amount of adrenaline to noradrenaline differed greatly from the normal ratio, especially during the marathon. The adrenaline percentage was 31 per cent for the runners, whereas the normal range is from 15 to 20 per cent. A slight increase in the ratio was observed also for the skiers; the mean ratio for these, 24.9 per cent, was, however, lower than for the marathon runners.

Owing to the small volume of urine excreted, the concentrations of noradrenaline and adrenaline in the urine were both high. Values as high as 75 μg (noradrenaline + adrenaline) per 100 ml were recorded and the direct injection of urine in the tests without prior extraction and concentration gave positive reactions as in cases of phaeochromocytoma, but it was established that some components in the urine interfered with the determinations and hence extracts were made in the usual manner and diluted for the biological evaluations. Owing to the high adrenaline contents in the extracts, it was in some cases necessary to dilute the extracts with physiological saline in the ratio of 1 : 20.

Table 20.

The urinary excretion of noradrenaline and adrenaline in 10 marathon runners and 10 long-distance skiers during competition.

Subject	Age years	Urine volume ml	Noradren. µg	Adren. µg	Adren. per cent	Competition
1	43	75	24.3	6.1	20.0	Marathon race
2	28	84	23.6	13.8	36.8	„
3	34	78	27.0	6.9	20.3	„
4	41	65	16.3	8.1	33.2	„
5	49	60	20.5	6.5	24.1	„
6	44	95	12.0	8.2	40.6	„
7	33	40	14.2	6.1	30.0	„
8	26	80	27.2	16.1	43.3	„
9	32	175	31.5	18.9	37.3	„
10	46	85	20.4	7.0	25.5	„
Mean	37	84	21.7	9.8	31.1	
1	30	125	25.0	10.9	30.4	40-km ski race
2	30	40	27.2	3.0	9.9	„
3	23	75	25.1	7.8	23.7	„
4	28	95	14.3	7.3	33.8	„
5	24	65	8.1	1.8	18.2	„
6	30	100	11.8	5.2	30.6	„
7	32	80	21.8	9.6	30.6	„
8	27	180	51.1	7.0	12.0	„
9	32	70	12.0	5.4	31.0	„
10	28	85	20.4	8.1	28.4	„
Mean	28	92	21.7	6.6	24.9	

C. HEAVY PHYSICAL WORK.

In recent years, national competitions have been arranged in Finland for woodcutters, to which contestants have been selected on the basis of regional competitions. The participants in the final competitions thus represent a group of selected skilful woodsmen. These competitions provide an unusual opportunity for studying the physiology of heavy physical exertion. In connection with the National Competition in 1954, the excretion of noradrenaline and adrenaline by some of the competitors was followed.

1. Material.

The original purpose was to examine the excretion by a larger number of competitors, but owing to the large area where they worked, it was not found practical to collect urine from more than five of the competitors. The noradrenaline and adrenaline contents were evaluated for urine samples collected not only during the competition, but also during the previous night, and during the afternoon and evening, and during the night following the competition.

The volumes of urine excreted during different periods were measured, and a 100-ml sample was taken from each and acidified to pH 3—4 with sulphuric acid. Until the final tests were made, the samples were stored at temperatures between 1 and 5° C.

2. Results.

The total amounts of noradrenaline and adrenaline found in the urine samples collected from each of the five competitors differed clearly from the normal owing to the highly increased excretion of noradrenaline and adrenaline during the competition. The amounts excreted during the night did not, however, deviate greatly from the amounts previously found for men (Table 21). The mean excretions for the five competitors during the night period were 7—8 μg of noradrenaline and 2.5 μg of adrenaline. The normal excretions found for men during an 8-hour night's rest are 5.7 μg of noradrenaline and 1.1 μg of adrenaline.

During the competition, which involved the cutting of firewood during a 6½—7-hour period, the amounts of noradrenaline and adrenaline excreted were very much increased. This was true for all the men, but the individual variation was great (Fig. 13). The greatest excretion of noradrenaline was recorded for a very big, robust man (V. V.). During the 7-hour period, his noradrenaline excretion was 182 μg and that of adrenaline 22 μg. The increase from the values for the night preceding and following the day of the competition was 35-fold, whereas the excretion during daytime is normally only about twice the night excretion. The excretion per minute for the whole 7-hour period was 0.44 μg of noradrenaline. The excretions were high also for the other competitors. The mean excretions for the 5 men during the 7-hour period

Table 21.

The urinary excretion of noradrenaline and adrenaline by participants in a woodcutting competition for periods before, during and after the competition.

Subject	Night preceding 23—7			Work period 7—14			Evening 14—23			Night following 23—7		
	noradr.	adr.	adr. %	noradr.	adr.	adr. %	noradr.	adr.	adr. %	noradr.	adr.	adr. %
A. V.	10.0	2.6	21.6	111.8	18.1	13.8	28.3	5.4	13.0	9.1	2.3	20.2
V. V.	5.2	3.2	38.1	182.2	21.6	10.6	13.6	4.5	24.9	6.0	3.0	33.3
M. V.	10.3	2.8	21.4	118.3	11.4	8.8	15.7	4.5	22.3	9.4	5.0	34.7
E. A.	6.9	1.2	14.8	61.0	9.9	13.8	3.5	1.2	25.5	8.4	1.8	17.6
U. N.				27.6	3.4	11.0	5.0	3.1	38.3	2.1	1.2	36.4
Mean	8.1	2.5	24.0	100.2	12.9	11.6	13.2	3.7	24.8	7.0	2.7	28.4

were 100.2 μg of noradrenaline and 13.2 μg of adrenaline, which are about 10 times the normal excretions during a similar period. Exceptionally low values were found for the participant (U. N.) who won the competition; he excreted 27 μg of noradrenaline and 3.4 μg of adrenaline during the competition. He was a man of slight build, but very agile. Also his excretion during the other periods was low, the figure for noradrenaline for the night following the competition, 2.1 μg, being clearly below the normal value.

During the afternoon and evening following the competition, when the competitors mainly rested, the amounts excreted were already clearly smaller than during the competition, but in most cases, however, greater than for normal subjects. The excretion during the following night had fallen nearly to the level for the night preceding the competition.

D. DISCUSSION.

Since the part of this investigation dealing with the effect of increased physical activity involved three widely different types of muscular exertion, running practice of short duration, long-distance running and skiing, and woodcutting in competition, these will be discussed separately.

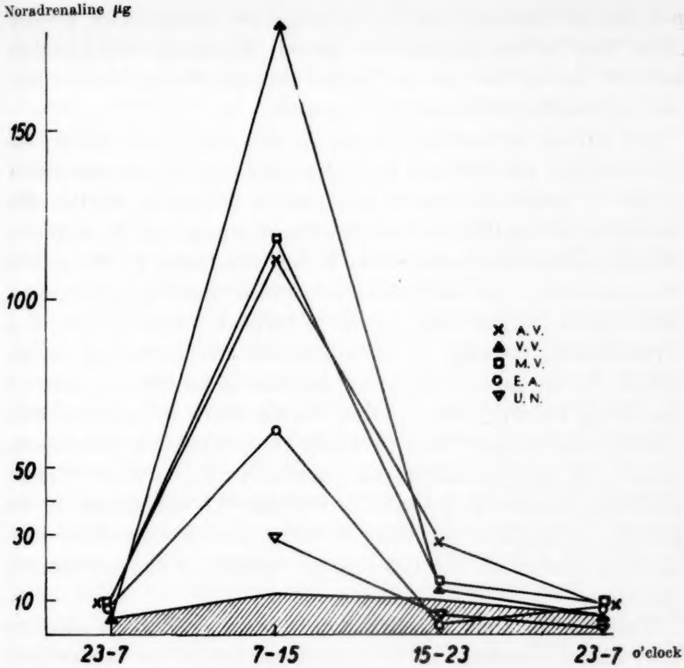

Fig. 13. Variation of noradrenaline excretion during day of competition in woodcutting. Five participants. See Table 21. Upper limit of shaded area denotes mean excretion for men under normal conditions (Table 16).

In the case of three athletes in training, in whom the noradrenaline excretion was followed by taking control samples of urine before as well as after the practice period, the subjects functioned as their own controls. In each case the periods over which the urine was collected were equal in length to the practice period. The noradrenaline excreted in urine was found to undergo a clear increase during the practice period in all subjects. This result is in agreement with the results of earlier investigators (Holtz et al. 1947, v. Euler and Hellner 1952, Holmgren 1956). It was also established that the increased noradrenaline excretion diminished very rapidly after the practice period ended. The mean excretion

per unit of time was slightly, although not significantly, greater after than before the practice period. Evidently noradrenaline secreted during the practice period and retained in the bladder was responsible for this slight increase.

The purpose of the experiments in which the excretion by the same athletes was followed during several practice periods over a period of about one month was also to determine whether the amount of adrenaline excreted in urine diminishes as the organism becomes accustomed to the stress. It has been found (v. Euler and Hellner 1952) that the muscular exercise must be greater in a well-trained subject than in an untrained person to effect a corresponding increase in the adrenaline and noradrenaline excretion. In the present study, no decrease in the amount excreted was noted, but evidently the time during which the subjects were studied was too short and obviously the subjects in question increased the exertion during the practice period as their physical conditions improved. In order to evaluate the significance of the practice, it would be necessary to follow the exertion under standardized conditions, but this was not possible in the experiments described.

The blood pressure is believed to be maintained at an adequate level during muscular work by the mediation of the baroreceptor reflex mechanism (v. Euler and Liljestrand 1946). During muscular work the blood vessels of the muscles widen and the peripheral resistance decreases. On the the other hand, the arterial blood pressure rises as a result of augmented cardiac output and compensating vasoconstriction in other regions (Bowen 1904, Paterson 1928, Holmgren 1956). In this regulation the sympathetic nervous system plays an important part, for it has been found by Freeman and Rosenblueth (1931) in cats, and Lord and Hinton (1945) and Holmgren (1956) in men that after sympathectomy the blood pressure diminishes during muscular work. Also under the influence of hexamethonium, a ganglion-blocking agent, the blood pressure falls during muscular work (Rønnev-Jessen 1953). It may be therefore assumed that the observed increase in noradrenaline excretion in urine during work is associated with increased activity of the sympathetic vasomotor nerve endings. v. Euler, Luft and Sundin (1955) and Sundin (1956) have shown that circulatory stress caused by tilting the

subject to an angle $+70 - +50$ degrees is accompanied by a definite increase in the excretion of noradrenaline as compared with the excretion when the subject is recumbent. FRANKSSON, GEMZELL and v. EULER (1954) and HALME, PEKKARINEN and TURUNEN (1956) have observed that the excretion of noradrenaline and adrenaline is often markedly increased during a few days following major operations. The augmented excretion in these cases can be assumed to be due to an increased activation of the vasomotor nerve endings to counteract the lowering of the blood pressure caused by factors associated with the operation.

In competitions of long duration of runners and skiers it was found that not only the noradrenaline but also the adrenaline excretion in the urine increases and the latter relatively more than the former. The mean adrenaline percentage was 31 per cent for the marathon runners, whereas the percentage for normal subjects was found to be 15—20. The percentage, 24.9, observed for the skiers also exceeded the normal figure. It is obvious that the secretion by the suprarenal medulla is appreciably increased by muscular exertion. This is apparent from the results of WADA, SEO and ABE (1935) who observed that during exhausting work the secretion of adrenaline by the suprarenals of dogs undergoes a large increase, while when no fatigue results from the work, it remains at the same level as at rest.

Metabolic factors probably influence the secretion of adrenaline during muscular exertion. The energy expenditure of the organism may increase to a level more than ten times the normal, since it is known that a well-trained marathon runner may consume 1300 kilocalories per hour (MOREHOUSE and MILLER 1953). Owing to the high consumption of energy, the store of carbohydrates decreases and the blood sugar level may diminish to 45 mg/100 ml during a marathon race (LEVINE et al. 1924). Hypoglycemia, on the other hand, increases the level of adrenaline in the peripheral and suprarenal blood and also in urine in man and in animals (HOLZBAUER and VOGT 1954, DUNÉR 1954, v. EULER and HELLNER 1952, PITKÄNEN 1956).

Also an oxygen deficiency in the organism, which results from exhausting work, may be expected to stimulate the suprarenal medulla. HOUSSAY and RAPELA (1953) have found asphyxia to increase the suprarenal secretion up to 34 times the normal in

dogs. Similar results have also been obtained in cats by KAINDL and v. EULER (1951). RAAB (1943) and also HÖKFELT (1951) observed a diminished adrenaline content in the suprarenals of rats exposed to low oxygen tension.

When the suprarenal gland of the dog was perfused with heparinized blood, a fall in the oxygen supply to the gland was found to lead to a rise in adrenaline output (BÜLBRING, BURN and DE ELIO 1948).

By evaluating the energy requirements, it has been found that woodcutting is one of the most exacting forms of work (TIGERSTEDT 1900, LUNDGREN et al. 1943, PYKE 1950). In their study of calorie intake in a woodcutting competition, KARVONEN and TURPEINEN (1954) concluded that the mean calorific value of the food consumed was 5500 Cal./day. When the change in weight was also included and calculated as fat, the calorie expenditure rose to 7800 Cal. per man during the competition day.

As expected, the excretion of noradrenaline and adrenaline in the urine was high during the competition for the woodcutters examined in the present study. An excretion of noradrenaline amounting to nearly 200 μg, which was observed for one of the woodcutters during the 7-hour working period, is so high that it would be clearly pathological under normal conditions. Nevertheless the excretion of noradrenaline by the woodcutters was in the normal range (2.1—10.3 μg) during the nights preceding and following the competition.

A high excretion over a period as long as this without any secreting tumours being present shows that the vegetative nervous system and the suprarenal medulla are able to produce these hormones at a high rate. If the same percentage (0.3—4.0) of noradrenaline is excreted in the urine during muscular work as is found when noradrenaline is given to normal subjects by infusion, we come to the conclusion that from 5 to 60 mg of noradrenaline may enter the blood stream during the 7-hour working period. The studies of ELMADJIAN et al. (1956 b) indicate that the percentage of noradrenaline measured in the urine after infusion is dependent upon the rate of infusion; when a larger dose was infused during a certain period, also the percentage that appeared in the urine was greater. The experiments were made on patients at rest and we do not know whether the same relationship applies between the

amounts of noradrenaline released in the organism by muscular work and excreted in the urine. It may also be suspected that owing to the higher metabolic rate during the muscular work also the decomposition of these amines proceeds much faster, and hence the amounts liberated in the organism may be much larger than those computed on the basis of urine analyses.

In none of the woodcutters was the percentage of adrenaline in urine higher during the work period than in normal persons and in this respect they clearly differed from the marathon runners. The absolute amounts of adrenaline excreted were, however, definitely higher than normal. Various factors such as the nature of the work and consumption of food, by which the blood sugar level was maintained, may have been responsible for the observed relatively low excretion of adrenaline as compared with that of noradrenaline.

IV

SUMMARY.

Method. An extraction method is described in which noradrenaline and adrenaline are adsorbed on aluminium oxide from urine adjusted to pH 8.5 and then eluted with sulphuric acid. It was established that if the adsorption is performed rapidly, very little inactivation of noradrenaline and adrenaline takes place at this pH. The method is rapid and 8—10 samples can be treated in one series. The adsorption is performed on unhydrolysed urine.

The recovery of noradrenaline and adrenaline added to the urine was approximately 73 per cent when this method of extraction was employed. The stability of the extracts was good; no reduction in biological activity was observed during a storage period of ten days at + 4° C.

In the study of the stability of noradrenaline added to urine, no variation in the analysed noradrenaline content occurred when the urine was stored in the cold (+ 4° C) at pH 6.5 and pH 4. The content did not change when the urine of pH 4 was stored two days at room temperature, but a marked decrease occurred when the pH of the urine was 6.5 during the storage at room temperature.

The biological activities of the extracts were tested on the blood pressure of the cat or rat and on the hen's rectal caecum. Hexamethonium was employed to increase the sensitivity of blood pressure reactions of the cats and rats.

Normal excretion. To evaluate the normal excretion of noradrenaline and adrenaline in human beings, the noradrenaline contents of 356 twenty-four-hour urine samples from 291 subjects and the adrenaline contents of 240 twenty-four-hour urine samples from 182 subjects were determined.

The subjects included 92 children,85 young and 57 middle-aged adults and 57 persons over 60 years of age.

In the group of children from 1.5 to 6 years old, the mean 24-hour excretion of noradrenaline in the urine was 5.6 μg and that of adrenaline 1.3 μg.

In the children from 7 to 16 years, the mean noradrenaline excretion was 14.5 μg per 24 hours and that of adrenaline 2.8 μg per 24 hours. The excretions in this group were approximately twice those in the preceding group, and a highly significant regression was established between the amount of noradrenaline excreted and the weight of the subject.

In the subjects from 17 to 29 years, the mean excretions were 24.5 μg of noradrenaline and 5.1 μg of adrenaline per 24 hours.

In the group from 30 to 59 years, the mean excretions were 25.2 μg of noradrenaline and 5.4 μg of adrenaline per 24 hours.

In the oldest subjects, whose ages varied from 60 to 96 years, the mean noradrenaline excretion was 23.1 μg and that of adrenaline 4.4 μg per 24 hours.

When the amounts of noradrenaline excreted were calculated per kilogram of body weight, it was found that the mean noradrenaline output was highest for both sexes in the group from 7 to 16 years old and decreased with increasing age. No differences in the 24-hour noradrenaline excretion were observed between the sexes when the mean excretions for the various age groups were compared.

No statistically significant differences in the urinary excretion of adrenaline calculated per kilogram of body weight were established between the different age groups and between the sexes.

The volumes of urine excreted by the children were large, but the mean noradrenaline concentration was low, only about one-half the concentration in adult urine. The amounts of noradrenaline excreted by 85 subjects from 17 to 29 years were not found to bear any relationship to the urine volume.

Diurnal variation. The mean noradrenaline excretion was 5.7 μg and that of adrenaline 1.1 μg in five men during an 8-hour night period, 11.9 μg of noradrenaline and 3.1 μg of adrenaline during the first 8-hour daytime period, and 10.1 μg of noradrenaline and 2.1 μg of adrenaline during the second 8-hour daytime period.

For three women, the mean excretions were 5.0 μg of noradrenaline and 0.7 μg of adrenaline during the night (8-hours), 10.8 μg of noradrenaline and 1.9 μg of adrenaline during the first daytime period, and 8.5 μg of noradrenaline and 1.5μg of adrenaline during the second daytime period.

For four children from 3 to 12 years old, the mean excretions during the 12-hour daytime period were 8.3 μg of noradrenaline and 1.4 μg of adrenaline and during the 12-hour night period 3.9 μg of noradrenaline and 0.5 μg of adrenaline.

Muscular work. Muscular work was found to effect very large increases in the amounts of noradrenaline and adrenaline excreted in the urine. The effect of 3 or 4 practice periods on the noradrenaline excretion was determined in three athletes over a period of one month. The amounts of noradrenaline excreted per unit of time during the practice period was from 4 to 6 times as great as the excretion before the practice period, but fell rapidly after the practice to the level before the practice period.

During exhausting muscular exertion of long duration (10 marathon runners and 10 skiers in a 40-km competition), the excretions of noradrenaline and adrenaline differed greatly from the normal. In an extreme case the noradrenaline excretion per unit of time was 17 times, and the adrenaline excretion 25 times, the normal. The mean ratio of adrenaline to total noradrenaline and adrenaline was 31 per cent for the marathon runners and 24.9 per cent for the skiers.

During a 7-hour period of muscular work of a very exacting nature (woodcutting competition), the mean excretion of noradrenaline in a group of five men was 100 μg. The amount of noradrenaline excreted by one of the competitors was 182 μg, which was thirty-five times the excretion by the same man during periods of rest.

V

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